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Carcinogenesis in the Mouse's Skin by the Infrequent Application at Long Intervals of Methylcholanthrene*

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This paper is a contribution to a group investigation on the early stages of experimental carcinogenesis organized by Dr. E. V. Cowdry. From a histological study of the early changes produced by a strong carcinogenic stimulus as represented by a 0.6 per cent solution of methylcholanthrene in benzene applied to a large area of skin 3 times a week, the conclusion was reached that the action of the carcinogen is not a direct stimulating effect inducing epithelial proliferation. Its direct effect is, on the contrary, a short toxic one lasting for several days, injuring the epithelial cells and inhibiting their mitotic activity. The subsequent epithelial proliferation which conveys the impression of a protective response can be accounted for adequately by assuming as a working hypothesis the formation in the skin of substances stimulating the cells to multiply over a prolonged period. For convenience of reference we shall call these hypothetical substances "proliferin."

This conclusion was based partly on the finding that a single application of methylcholanthrene is capable of inducing in a small percentage of a group of mice an active epithelial proliferation which persists for many weeks producing a massive hyperplasia, while it fails to do so in other animals of the same strain subjected for a period of 1 or 2 months to frequently repeated applications of the carcinogen (Figs. 1 and 2). The detailed evidence on which these conclusions are based is now being prepared for publication.

If the carcinogen had a direct stimulating effect on the epithelium, as is generally believed, then the degree of the epithelial hyperplasia should be a direct function of the amount of the carcinogen applied. This is obviously not the case when a single application of the carcinogen can produce in some animals a high degree of hyperplasia while in others frequently repeated applications fail to elicit a hyperplasia. But this phenomenon can be accounted for on the alternative conception mentioned above. In that case the applications of a strong carcinogen repeated frequently at short intervals of time, as used in the conventional technic of experimental carcinogenesis, may produce an inhibition sufficiently effective to overcome the proliferative stimulus due to the hypothetical proliferin. While the ultimate proof of this conception lies in the identification of that substance, it is possible to test experimentally the validity of this view by spacing the applications of the carcinogen at longer intervals. This would allow the stimulating effect of proliferin, which from our observations persists over a long period when once formed, to continue for a longer period without being counteracted by the toxic inhibitory effect of the carcinogen, which is more transient.

MATERIALS AND METHODS

We carried out, therefore, 3 series of experiments on mice of the Swiss strain, using a method of application which in previous experiments with frequently repeated applications had been found to give a near optimum effect; namely, a 0.6 per cent solution of methylcholanthrene applied to a large area of skin by a single brush stroke from the nape of the neck for a distance of about 1.5 cm. By applying 10 similar brush strokes to a piece of weighed filter paper it was found that the average amount of methylcholanthrene applied to the skin at each brush stroke is 0.1 mgm. Using less absorbent typing paper the amount delivered at each brush stroke was 0.07 mgm. In the standard technic this treatment is applied 3 times a

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FIGS. 1 AND 2

week for 14 weeks; *i.e.,* 42 applications, when all painting is stopped. By this standard method 100 per cent of the mice develop skin cancer, the first malignant tumor appearing in the 6th week; *i.e.,* after 15 applications; the last in the 24th week.

In order to test the results of prolonging the intervals of application, the carcinogen was applied once every 2 weeks to a series of 10 mice (Series I) of which one died early in the experiment. In another series of 12 mice it was applied once every 3 weeks (Series II). The results being successful, a third experiment was begun with 50 mice which received one application once a month (Series III). Although this third experimental series is still in progress, the results obtained after 7 applications are sufficiently striking to make possible a comparison with the standard technic. For convenience of reference, the method of infrequent painting at long intervals just outlined will be called the protracted technic.

RESULTS

The results are given in Table I. There is obviously an essential difference between the standard and the protracted method of application in the selection of the factors by which the carcinogenic effect of the hydrocarbon is determined. In the standard method it is measured by the ratio of the number of cancerous mice, expressed in percentages, to the time necessary to induce cancer, reckoned from the first exposure to the carcinogen; *i.e.,* the latent period. In the protracted method this time factor of exposure to the carcinogen is disregarded and the carcinogenic effect is determined by the ratio of the number of cancerous mice to the effective carcinogenic dose of the carcinogen applied, as shown in Table II. This is the usual method to determine a direct biological effect of a chemical substance.

Table II shows that in all the three series treated by the protracted method, the carcinogenic potency of the hydrocarbon is higher, or, if expressed in terms of its effective dose, the effective dose is smaller than in the standard method. Thus, in Series I the dose necessary to induce cancer in all the animals, 1.4 mgm. in 14 applications, is equal to that applied in a month's painting by the standard method (3 times weekly). In Series II, it is equal to the dose applied in 3 weeks' painting by the standard method. In Series III, which

DESCRIPTION OF FIGURES 1 AND 2

FIG. 1.—Slight epidermal proliferation of mouse skin painted thrice weekly for 52 days (17 paintings) with 0.6 per cent methylcholanthrene in benzene. Axial section. Mag. × 12.

FIG. 2.—Marked epidermal proliferation in mouse skin 28 days after a single application of the same carcinogen. Axial section. Mag. × 12.

at the time of writing has only reached the stage of 7 monthly applications, 50 per cent of the mice have developed cancer in response to a dose of carcinogen equal to that applied in only 2 weeks' painting. No such results have been obtained by us after a corresponding number of applications by the standard method.

The experimental Series I and II were of a preliminary nature and were, therefore, carried out on a small number of animals, so that a comparison between these two series does not give differences which are statistically significant. The experiments are being repeated on a larger number of animals. However, a comparison between these two series with Series III shows that the effective carcinogenic dose of the same hydrocarbon varies with the intervals of time which elapse between successive applications. The effective dose diminishes as the interval between two successive applications is prolonged, and this is true whether one takes the minimal dose after which the first carcinoma appears or the dose inducing cancer in 50 per cent of the survivors. For the incompleted Series III with an interval of 1 month between successive applications it can be said at present that both the minimal cancer-producing dose and the dose necessary to induce cancer in 50 per cent of the animals are even smaller than in either Series I or in Series II.

Although the time of exposure to the carcinogen is disregarded in this method as a factor determining the carcinogenic potency of a hydrocarbon, the latent period, *i.e.*, the time at which cancer appears, shows an interesting relationship. The latent period for the first carcinoma in all three series is longer than in the standard method, where with our technic it was found to lie between the 6th and 8th week. In the first two series there is a similar interval of time necessary to induce cancer in the whole group. After 27 weeks cancer had developed in all the animals of Series II and in 8 out of 9 animals in Series I. This is again longer than the length of the maximal latent period in the standard method, about 24 weeks. In the incompleted Series III the time necessary to induce cancer in all the mice will be even longer than Series I and II.

Table I: Development of Skin Cancer by the Protracted Method in Relation to the Dose of Methylcholanthrene Applied

No. of applications; also carcinogen in 0.1 mgm.	Series I Once in 2 weeks		Series II Once in 3 weeks		Series III Once in 4 weeks	
	No. of mice with cancer	Latent period in weeks	No. of mice with cancer	Latent period in weeks	No. of mice with cancer	Latent period in weeks
1	0	..	0	..	0	..
2	0	..	0	..	0	..
3	0	..	0	..	2	13
4	0	..	0	..	5*	17
5	0	..	1	15	7	21
6	0	..	2	18	12	25
7	0	..	6	21	9*	29
8	0	..	2	24	..	..
9	0	..	1	27	..	..
10	4	20	..	..	..	..
11	1	22	..	..	..	..
12	1	24	..	..	..	..
13	2	26	..	..	..	..
14	1	33	..	..	..	..
Total no. of mice with cancer	9		12		35†	
Total negatives	0		0		15†	

* One sarcoma included.
† Experiment not yet completed.

Table II: Comparison of the Total Dosage of Methylcholanthrene Required to Induce Cancer by the Standard and Protracted Technics

Frequency of painting	Methylcholanthrene applied before appearance of cancer, in mgm.		
	In first mouse	In 50 per cent of mice	In 100 per cent of mice
Standard technic			
Three times per week	1.5	4.2	4.2
Protracted technic			
Once in 2 weeks	1.0	1.1	1.4
Once in 3 weeks	0.5	0.7	0.9
Once a month	0.3	0.6	Not yet determined

Discussion

These results are not in accordance with the accepted view that the carcinogenic hydrocarbons induce cancer in the skin by stimulating directly the mitotic activity of the epithelium. They support the conception, arrived at from a histological study of the early changes in carcinogenesis, that the carcinogenic hydrocarbons produce a transient toxic effect on the epithelium, inhibiting mitotic activity, and that the epithelial proliferation which eventually leads to cancer is due to the formation in the skin of a substance stimulating the epithelial cells to mitotic activity for a prolonged period of time. Sometimes this proliferation is so prolonged that even a single application of the carcinogen is sufficient to induce cancer. In a group of 6 Swiss mice subjected to a single application, one surviving animal developed a skin carcinoma after an interval of 6 months. A similar result has been recorded by Mider and Morton (1) working with the C57 brown strain. Another group of 14 Swiss mice received one application of methylcholanthrene 4 months before the time of writing. In this group one animal has already developed cancer (sarcoma) after 3 months, which is the shortest time recorded for a single application.

Bearing in mind that, in terms of biological time, a month of a mouse's life corresponds to 2 years of human life, these results have an important bearing on the etiology of cancer in man by demonstrating that a continued exposure persisting over a long period of time is not essential for the development of a malignant epithelial tumor, as is generally supposed. A few isolated exposures to a strong carcinogenic stimulus separated from each other by long intervals of time can be effectively carcinogenic. At present the efficacy of the protracted method of carcinogenesis has been demonstrated only for the experimental conditions defined in this paper; namely, the application of a highly effective carcinogenic solution to a large area of skin.

Summary and Conclusions

Cancer has been induced in the skin of mice by a method of application in which the carcinogen acts on the cells infrequently and at long intervals. In this method the carcinogen was applied at intervals of 2 weeks, 3 weeks and of 1 month, respectively. By this technic the carcinogenic potency of an agent is measured in terms of the effective dose of the carcinogen; that is, the dose which induces cancer. In the standard method of continuous application, the carcinogenic potency is measured by the time necessary to induce cancer. Using the protracted technic, it was found that the effective carcinogenic dose is smaller than that found by the standard technic, and further that the dose becomes increasingly smaller as the interval between each successive application is prolonged.

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Further Study of Blastomogenic Substances in the Human Body*

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The purpose of this report is to present the results of two additional series of experimental demonstrations of the occurrence of endogenic blastomogenic (carcinogenic) substances in the organs of human beings who had died of malignant tumors.

Review

In January, 1937, Schabad (11) showed that benzol extracts of the liver of a patient who had died of cancer were carcinogenic when injected into mice. Malignant and benign tumors were produced at the site of injection or in a remote location. In 1938, Neufach and Schabad (9, 10) proved that a benzol extract of the bile of patients who had died of cancer likewise possessed a blastomogenic effect. Recently, in 1939 and 1940, Kleinenberg, Neufach, and Schabad (4, 5, 6) summarized the results of a large number of experiments aimed at studying the effect of extracts obtained from organs of patients who had died of cancer and from persons who had died of other diseases. The results showed that extracts of organs from patients dying of cancer produced a greater number of tumors than extracts of organs of persons dying of other causes.

The presence of blastomogenic substances in the organs of patients who had died of malignant tumors was established by this work and the assumption was made that these substances found in the human body were of endogenic origin. These results were confirmed in 1940 by des Ligneris (1), Hieger (2), Menke (7), Steiner (18), and Tanaka, whose report is included in a paper by Kinosita (3).

Additional detailed accounts of methods of extraction and results of tests for carcinogenicity of the extracts have been published by Neufach (8, 9), and by Schabad (11-16).

Carcinogenic Extracts of Human Liver and Bile

At present we are attempting to make a systematic study of the nature of the blastomogenic substances found in the human body. The first question with which we were confronted was whether or not it was possible to extract from human organs chemical compounds which produced tumors. Indeed, the crude benzol extracts obtained from the liver or the bile, which in our previous experiments had produced a large number of tumors in mice, were a mixture of various substances, mostly lipids. The usual procedure of fractioning lipid extracts from organs and simultaneously testing the biologic effect of each fraction is in this case made difficult by the fact that the results of testing the supposed biologic effect can only be obtained on a large number of animals and after a long period of time, up to 2½ years; *i.e.*, until the natural death of the mice under experiment. Therefore, we were obliged to confine our investigations to the study of one stage of fractionation. To begin with, we decided to study that fraction of the extracts obtained from the liver of cancerous patients which contains nonsaponifiable substances. For the purpose of obtaining this fraction we employed the following method.

The capsule of the liver was removed and the liver washed free of blood. It was then carefully passed through a mincing machine and crushed with anhydrous sodium sulfate. Three successive extractions were then made from the mass of liver tissue by the use of previously distilled benzol, the volume of the latter being in each case twice the weight of the liver. The extracts thus obtained were mixed together and the benzol removed by distillation under reduced pressure at a temperature of about 40° C. The residue of the benzol extract was a brown-colored oil containing crystals of glycerides and giving a positive Libermann-Burchard test. The weight of the extract varied from 11.38 to 212.53 gm. (Table I), thus amounting on the average to 4.7 per cent of the weight of the raw liver. The preparation was then subjected to saponification by means of a 2N alcoholic solution of potassium hydroxide for 24 to 48 hours on a boiling water bath. After the saponified material thus obtained was diluted by five-fold its volume of water, with an addition of some crystals of sodium chloride, three successive extractions of the nonsaponifiable fraction were made by means of sulfuric ether. The ether extracts were then mixed together and condensed *in vacuo* to a volume of 150 to 200 cc. and successively washed with N KOH, water, N HCl, and water again, until the water used for washing showed a neutral reaction to litmus. The orange-brown solution thus obtained was perfectly transparent. The ether was then

* On account of the difficulties of communication, created by the war, it was not possible to submit the editorial revision of this paper to Professor Schabad.—Editor.

distilled. The remaining nonsaponifiable fraction presented an orange-brown mass which was partly amorphous, partly crystalline, and in which the test for cholesterol was markedly positive. When radiated with ultraviolet rays through Wood's filter the mass in solution showed a bluish-violet fluorescence. The nonsaponifiable fraction varied in weight from 0.259 to 8.25 gm. (Table I) making up on the average 4.8 per cent of the weight of the benzol extract.

As can be seen from Table I, we investigated 9 human livers which had been obtained from 2 male and 7 female patients, varying in age from 37 to 69 years, who had died of different types of cancer in several locations:—in 6 cases, cancer of the stomach; in 1, primary cancer of the liver; in 1, cancer of the uterus; and in 1, cancer of the ovary. Out of the 9 cases of cancer, 4 patients had numerous metastases in the liver and 1 patient a primary tumor of the liver. Thus, in 5 cases out of the 9, preparations were more readily made of the tumor tissue than of the liver tissue itself. Finally, it should be noted that in 2 of the cases the primary tumor had been removed by means of surgical interference a long time before the patients' deaths and that in one of the latter (Table I, No. 2) death had been due to recurrence and metastases.

We carried out 9 experiments using 50 mice, of which 23 were of our R.V. strain and 27 of a strain that had not been previously studied. The nonsaponifiable fraction was diluted in from 4 to 8 cc. of olive oil and injected subcutaneously from 3 to 8 times, always in the same spot, the interval between the injections being from 10 to 15 days.

The results obtained are shown in Table II which includes for the sake of comparison data concerning the number of spontaneous tumors observed in mice of the R.V. strain, as reported by Schabad and Kleinenberg (17).

As can be seen from Table II the nonsaponifiable fraction under investigation was found to produce a number of tumors in mice which were two to three times more frequent than those observed to occur spontaneously. Thus, for instance, tumors were found to occur in 21.7 per cent of the total number of mice of the R.V. strain which were subjected to the experiment and in 31.2 per cent of the mice which had reached an age of over 8 months. In two of the cases malignant tumors of the jaws were observed to de-

Table I: Data on Liver Extracts

Exp. No.	No. of the liver	Sex	Age	Diagnosis	Weight of liver in gm.	Weight of benzol extract in gm.	Weight of nonsaponifiable fraction in gm.
1	80	F	54	Cancer of the stomach with metastases to the liver	1,000	212.53	8.04
2	112	F	43	Cancer of the ovaries with metastases to the liver *	2,400	122.50	6.75
3	138	M	49	Cancer of the stomach with metastases to the liver	1,540	11.38	0.259
4	143	M	65	Cancer of the stomach with metastases to the liver †	2,160	19.75	0.75
5	166	F	50	Cancer of the uterus	1,050	121.30	8.25
6	180	F	58	Cancer of the stomach, fibromyoma of the uterus	790	27.14	0.95
7	184	F	55	Cancer of the stomach	800	25.98	0.88
8	190	F	37	Cancer of the stomach ‡	800	....	0.95
9	214	F	44	Cancer of the liver (primary)	1,400	31.02	0.60

* Tumor had been removed a long time before.
† Tumor had been removed 9 years previously.
‡ Extraction made with alcohol.

Table II: Induction of Tumors in Mice by Injection of Liver Extracts *

Strain of mice used	Total no. and sex	No. reaching over 8 months of age	Total no. with tumors	No. with malignant tumors	Tumors at site of injection	Tumors of the jaws	Tumors of the lungs
Unknown origin	27 (16 ♂, 11 ♀)	13 (7 ♂, 6♀)	3	1	1	..	2
R.V. strain	23 (11 ♂, 12 ♀)	16 (6 ♂, 10 ♀)	5	3	..	2	3(1 malignant)
Control: spontaneous tumors in R.V. strain	634 (304 ♂, 330 ♀)	389 (192 ♂, 197 ♀)	40	9	..	..	33(4 malignant)

* Professor Schabad's text of this Table contained percentages for many of the different entries. These have been omitted for simplification.—Editor.

velop, and in three cases, tumors of the lungs, one of them being a cancerous tumor. In the experiments made on mice of unknown origin tumors were found to occur 3 times; *i.e.,* in 11.1 to 23 per cent of the cases. Among these tumors, the one deserving particular notice was a sarcoma which had developed in a male mouse at the site where the fraction of the extracts had been injected. This tumor had been produced as a result of injecting the benzol extract obtained from the liver of a male patient, aged 65, who had died of cancer of the stomach (Table I, No. 4). The nonsaponifiable fraction weighing 0.75 gm. was given in 3 successive injections in a solution of 8 cc. of olive oil to 7 three-month old mice, out of whose number only 2 reached the age of over 8 months. In 5 to 6 months after the beginning of the experiment one of the mice was found to have a tumor on the site of the injections which in a short time attained 2×2.5 cm. in size. A microscopic study of the tumor revealed a typical polymorphous sarcoma, showing infiltrative growth into the neighboring tissues. A cavity in the center of the tumor contained some remains of the injected substance.

Thus, injections of the nonsaponifiable fraction of benzol extracts obtained from the liver of patients who had died of cancer in some cases produced tumors in mice. It should be noted that in one instance we succeeded in producing a sarcoma at the site where the fraction under test was injected and that the total number of tumors obtained exceeded by 2 to 2.5 times the incidence of spontaneous tumors observed in mice of the R.V. strain.

The method of obtaining the nonsaponifiable fraction employed by Hieger (2) and Steiner (18) differed from ours in that they subjected to direct saponification all the tissue of the liver and subsequently extracted the nonsaponifiable fraction. In contrast, we confined ourselves to obtaining the nonsaponifiable fraction from a benzol extract of the liver, as our previous investigations had shown it to contain blastomogenic substances. Furthermore, by saponifying the lipid fraction alone we avoided the possibility of obtaining a number of "ballast" substances.

In taking up our investigations with the object of discovering blastomogenic substances in the human body and starting on the assumption that they might be of endogenic origin, we already had at our disposal a number of data concerning exogenic substances obtained synthetically. As a result of a large number of experiments made with the benzol extracts obtained from the liver and the bile, we acquired certain new data concerning the blastomogenic substances we had discovered in the human body. In particular, we found that these substances were soluble in organic solvents and fats, that they were resistant to heating up to a temperature of 140° C., that they were not liable to decompose under the effect of air and could remain intact for a long period of time in a benzol solution. In addition, the present investigation has served to show that endogenic blastomogenic substances, or at any rate some of them, belong to the group of nonsaponifiable substances. As a consequence of these findings, we are now able to continue the study of endogenic blastomogenic substances by further investigating the nonsaponifiable fraction.

Carcinogenic Extracts of Human Lung

It was but natural to pass from the study of extracts of liver and bile to the study of the effect produced by extracts obtained from the tissues of other organs of the human body. In the first place our attention was drawn to the lungs. On the one hand, the lungs seem to play a certain part in the exchange of lipids which may serve as solvents for blastomogenic substances. On the other hand, primary tumors produced by injections of exogenic blastomogenic substances are known to develop frequently in the lungs of the mice under experiment, a fact which can be accounted for by the circulation of these substances throughout the animal organism, as reported by Schabad (15).

The materials used in the present investigation were the lungs of persons who had died of malignant tumors (Table III), as well as those of persons who had died of other diseases, and had never been affected with tumors. The latter thus served as controls (Table IV).

As can be seen from Table III we had at our disposal 19 cases of malignant tumors, 8 of which were cancer of the stomach. The other patients were affected with cancers of different location and one of them had a sarcoma. In only 3 of these cases were the lungs used in our experiments found to contain tumorous tissue (2 cases affected with primary cancer of the lungs and 1 case having sarcomatous metastases). The lungs used as control were those of 20 persons who had in most cases died of pneumonia. With respect to sex the persons whose lungs were used as control fully corresponded to the cancer patients under investigation, while as regards their age those who had died of cancer were somewhat younger than the persons whose lung extract had served as control.

Extracts from the lungs as well as those from the liver were prepared according to the method worked out at this laboratory and described by Neufach (8). The extracts obtained were an oily liquid, light brown in color and containing crystals. The weight of the extracts varied from 1.7 gm. to 14.15 gm., averaging 5.47 gm. The number of mice used in the experiment

was 212, 111 of which were animals of the R.V. strain and 101 of unknown origin.

None of the extracts was mixed. Each was used in a separate experiment. The extracts were diluted with olive oil and injected subcutaneously into mice in the same spot from 4 to 10 times at intervals of 10 to 20 days. The results obtained are shown in Table V.

As can be seen from Table V a large number of the mice which were given injections of extracts obtained from the lungs of persons who had died of malignant tumors were affected with tumors. Thus, out of the 46 mice of the R.V. strain which had reached the age of over 8 months, 25 mice; *i.e.*, 54.3 per cent of the total number, had some kind of tumor. Malignant tumors occurred in 6 mice (13.8 per cent). The fact that these data coincided with the results obtained by injecting liver and bile extracts, which produced tumors in 50.0 to 57.4 per cent of mice having reached the age of over 8 months (6) deserves special notice. Extracts from the lungs of persons who had never had any tumors produced a much smaller number of tumors in mice; namely, in only 13.6 to 23.1 per cent of the cases instead of the previously mentioned 37.3 to 54.3 per cent. Nevertheless, their number considerably exceeded that of tumors occurring spontaneously in mice of the R.V. strain (6.3 to 10.2 per cent).

It should be noted that in one case we observed a sarcoma which developed at the site of injection of an extract of the lungs of a woman, aged 89, who had been affected with cancer of the gall bladder but had no metastases in the lungs. Five gm. of the extract were diluted in an equal volume of olive oil and administered to 4 mice of the R.V. strain in 6 injections of 0.3 to 0.4 cc. each. In 17 months' time after the beginning of the experiment, the female mouse which was the last to survive developed a spindle cell sarcoma at the site of the injections. Another mouse of the same series, which died 16 months after the start of the experiment, had a squamous cell cornifying carcinoma of the mouth cavity.[1]

TABLE III: CASES OF DEATH CAUSED BY MALIGNANT TUMORS

Kind of tumor	No. of cases	No. with metastases in lungs	No. without metastases in lungs	Sex		Age		
				Male	Female	21 to 40	41 to 60	61 to 80 and over
Cancer of the stomach	8	..	8	5	3	1	4	3
Cancer of the lungs	2	..	2 *	2	..	..	..	2
Cnacer of the larynx	2	..	2	2	..	..	1	1
Cancer of the rectum	2	..	2	1	1	..	2	..
Cancer of the gall bladder	1	..	1	..	1	..	..	1
Papilloma of the bladder	1 †	..	1	..	1	..	..	1
Hypernephroma	2	..	2	2	..	..	..	2
Sarcoma of the skin	1	1	..	1	..	..	1	..
Totals	19	1	18	13	6	1	8	10

* The extract was made from combined lung and tumor tissue.
† Death due to hemorrhage.

TABLE IV: CASES OF DEATH NOT CAUSED BY MALIGNANT TUMORS

Diagnosis	No. of cases	Sex		Age		
		Male	Female	21 to 40	41 to 60	61 to 80 and over
Pneumonia	12	7	5	4	2	6
Primary cirrhosis of the kidney and hypertonia	3	2	1	..	2	1
Thrombosis of the coronary arteries	1	1	..	..	..	1
Traumatic shock	2	2	..	2	..	..
Purulent otitis	1	1	..	1	..	..
Pyelocystitis	1	..	1	..	..	1
Totals	20	13	7	7	4	9

Experiments with extracts obtained from the lungs of persons who had died of tumors were found to produce carcinomas of the mouth cavity in 3 cases, while experiments made with extracts of lungs of persons dying of other causes failed to produce such tumors, even in mice of the R.V. strain.

The greatest number of tumors in all the series occurred in the lungs. These tumors were benign adenomas, with one exception, which was a carcinoma. In the experiments with extracts of lungs from patients who died of cancer, tumors of the lungs occurred in 31.3 to 47.8 per cent of the cases, while in the

[1] The extract obtained from the liver of the same person was also found to possess a marked blastomogenic effect as shown by the results of our previous experiments.

TABLE V: INDUCTIONS OF TUMORS IN MICE BY INJECTIONS OF BENZOL EXTRACTS OF LUNGS *

Conditions of the experiment	Total no. of mice	No. of mice 8 months old or more	Total no. of mice with tumors	No. of mice with malignant tumors	Tumors on the site of extract injection	Tumors of skin and sebaceous glands	Tumors of the jaws	Tumors of the liver	Tumors of the lungs	Tumors of the thymus
Extracts from lungs of patients who had died of cancer injected in R.V. strain	67 (42 ♂, 25 ♀)	46 (32 ♂, 14 ♀)	25	6	1	1	3	1 (hepatoma)	22 (1 developed before 8 months of age)	1 (before 8 months of age)
Extracts from lungs of patients dying of other causes	44 (25 ♂, 19 ♀)	26 (15 ♂, 11 ♀)	6	1	..	..	..	..	6 (1 cancer)	..
Control: Spontaneous tumors in mice of R.V. strain	634 (304 ♂, 330 ♀)	389 (192 ♂, 197 ♀)	40	9	..	1 (cancer of the skin)	..	1 (hepatoma)	33 (4 cancer)	..
Extracts from lungs of patients who had died of cancer injected into mice of unknown origin	28 (18 ♂, 10 ♀)	15 (9 ♂, 6 ♀)	3	..	..	..	..	..	3	..
Extracts from lungs of patients dying of other diseases injected into mice of unknown origin	73 (40 ♂, 33 ♀)	32 (17 ♂, 15 ♀)	4	..	..	..	..	.	4	..

* See footnote to Table II concerning omission of percentages.—EDITOR.

experiments with extracts of lungs of persons dead of other diseases, they developed only in 13.6 to 23.2 per cent of the mice. In the R.V. strain the same kinds of tumors occurred spontaneously in 5.2 to 8.48 per cent of the mice. In experiments carried out on mice of unknown origin, only adenomas of the lungs were produced. These tumors were twice as frequent following injections of extracts of lungs of cancerous patients as after injections of noncancer extracts.

Thus, the extracts obtained from the lungs of persons who had died of cancer were found to produce various tumors, both benign and malignant, in a number of mice, either at the site of extract injection or, more frequently, at some distance from it. Extracts from the lungs of persons who had died of other diseases than cancer also produced tumors in greater frequency than they occurred spontaneously in our R.V. strain. The number of tumors produced by extracts obtained from the lungs of patients affected with cancer was 2½ times greater than that of tumors observed to occur after the administration of extracts from the lungs of patients who had died of other diseases.

The results obtained on mice of different origin confirm, on the whole, the observations made with respect to mice of the R.V. strain. It should be particularly noted that benzol extracts from the lungs had an effect analogous to that produced by the liver extracts previously studied. It is of interest to note that both the investigations carried out with liver extracts and the experiments of the present series have shown that extracts obtained from an organ devoid of tumorous tissue possess a blastomogenic effect.

Discussion, Summary, and Conclusions

The results obtained in these experiments demonstrate that blastomogenic substances, which can be extracted by means of benzol, occur not only in the human liver but also in the human lungs. This fact, on the one hand, confirms all the earlier data obtained by us as regards the blastomogenic effect of extracts obtained from human organs, and extends the material previously reported. On the other hand, a study of the extracts obtained from the lung tissue contributes towards elucidating the nature of the blastomogenic agents contained in the human body, as it leads to the exclusion of the action of a number of other substances peculiar to the liver tissue, such as, for instance, a large quantity of pigments, bile, acids, etc. Finally, it is impossible to overlook the fact that the extracts obtained from the lung tissue were far less toxic and gave rise to general irritation to a lesser extent than either liver or bile extracts. In spite of this, however, the number of tumors they produced was no smaller. This fact speaks in favor of the specificity of the effect of the blastomogenic agents studied by us.

At present we are not yet able to decide whether the observed blastomogenic effect of the extracts obtained from the lungs is due: (a) to their containing blastomogenic substances of exogenic origin which might have, for instance, penetrated from the air; (b) to the origination of endogenic blastomogenic substances in the lungs; or (c) to the penetration into the lungs of endogenic blastomogenic substances which had originated in some other organ (*e.g.* the liver) and had been conveyed into the lungs by the circulation. However, in connection with the questions raised, it should be emphasized that there was a very close coincidence between the results of the effect produced by extracts obtained from the lungs and by those obtained from the liver, which seems to speak in favor of the blastomogenic agents found in the lungs being of endogenic origin. Finally, the markedly different effects produced by extracts obtained from the lungs of persons who had died of cancer and by those obtained from persons who had died of other diseases seems likewise to speak in favor of the endogenic origin of the blastomogenic substances found in the human body, as both groups of patients had an equal chance of having been subjected to the aerogenic action of exogenic blastomogenic agents.

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On the Mechanism of Action of Carcinogenic Substances*

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In the study of cancer one of the central problems is the elucidation of the pathogenesis of malignant tumors. The direction taken by such studies varies in accordance with prevailing points of view on the etiology of cancer. Since the discovery of carcinogenic compounds by Cook, Kennaway, and others (1, 7), the theory of a chemical etiology of malignant tumors has been advanced. According to this theory the so-called "spontaneous" tumors, which include the majority of the tumors of man, and which are of the greatest interest for practical medicine, develop as the result of the action of endogenous carcinogenic substances engendered in the organism. Recently the hypothesis of endogenous carcinogenic substances has been strengthened by the results of the investigations of Schabad (44, 45), Hieger (17), des Ligneris (9, 10), Steiner (47), and Kleinenberg *et al.* (19). A tendency exists also, as indicated by the work of Roffo (43), to invoke a chemical explanation for the carcinogenic action of certain physical factors, such as ultraviolet rays. There is reason to believe that certain endogenous carcinogenic substances may prove to be closely related to some of the known synthetic carcinogenic hydrocarbons.

If the theory of the chemical etiology of malignant tumors be accepted as a working hypothesis, one of the first questions to arise concerns the mechanism of action of carcinogenic substances. What is their mode of action? In what way do these substances affect normal cells to transform them into tumor cells?

Problems of the mechanism of the action of carcinogenic substances have been of particular interest to these laboratories in recent years. The purpose of this communication is to present and discuss the results of some of the studies in this field which I have conducted in association with collaborators.

* Editorial Note: On account of conditions caused by the war, it has not been possible to communicate with Professor Larionow without long delays. His manuscript was revised and partially rewritten by the Secretary of the Editorial Committee.

Oxidation Processes in the Organism

If it is agreed that the cells in malignant tumors possess a special metabolism, the principal characteristic, according to Warburg, being a disturbance between respiration and glycolysis, the surmise arises that carcinogenic hydrocarbons may effectuate these changes in tissue respiration and carbohydrate metabolism at the point of their application. On the other hand, the work of Fischer-Wasels (15) and Neumann (34, 35) indicates that carcinogenic agents may produce a disturbance of respiratory and glycolytic processes in organs, as an essential factor of the so-called "general cancer predisposition."

To obtain information on these possibilities we investigated the oxidation processes occurring in the animal organism. M. Tchertkova studied the ratios of carbon to nitrogen and of "vacate-oxygen" to nitrogen in the urine, which Müller (31-33) and Bickel (2, 3) regard as ratios characteristic of the state of oxidation processes occurring in the body. The investigations were carried out on 90 mice and 7 rabbits. During a period of 4 months, the skin of mice in the interscapular region was painted every third day with a 0.3 per cent solution of benzpyrene in benzene. Papillomas appeared toward the end of this period and during the next 2 months after the cessation of the painting carcinomas developed. Urine was collected from a group of 10 mice and analyzed. Carbon was determined by the method of Osuca (38, 39). For nitrogen, the determinations were made by the Kjeldahl method and the vacate-oxygen was calculated by the method of Müller (31-33). Urine of normal mice and of mice painted with the solvent, benzene, was used as control. The mean values are presented in Table I.

The data presented in Table I show that throughout the period of painting with benzpyrene, until the appearance of papillomas, the oxidation coefficient remained unchanged and did not differ from the coefficient found in mice painted with benzene and in

normal mice. The ratio of C:N did not change during the period of painting.

A distinctly different picture came to view after carcinoma had been produced. M. Tchertkova found that in mice, soon after the origination of skin carcinoma, the values for the ratios of C:N and vacate-O:N in the urine increased. The values are presented in Fig. 1. They indicate that the oxidation processes had deteriorated.

From Fig. 1 it is seen that the values of these coefficients in mice with carcinomas exceed the limits of the normal variations, while the values for mice with papillomas lie more frequently within the limits of normal variations. In some of the mice in which higher values of these coefficients occurred, the initial stages of carcinomas existed, together with papillomas, as shown by histological sections of the lesions. Frequently, in the course of applications with benzpyrene, the change from papilloma to carcinoma takes place very rapidly. As a rule our diagnoses of such changes were made macroscopically because we needed the mice for further investigations.

TABLE I: MEAN VALUES OF MÜLLER'S COEFFICIENT; VACATE-O:N

Period of painting in months	Benzpyrene	Benzene	Normal mice
1	1.0	1.3	1.1
2	1.0	1.1	1.0
3	1.1	1.1	1.2
4	1.2	1.2	1.3

Rabbits were painted with benzpyrene every second or third day for a period of 7 months, the solution being applied to the anterior surface of the ears. The only local changes noted were loss of hair and hyperkeratosis. The urinary ratios of C, N, and vacate-oxygen during the painting period varied within the same limits as those determined before the carcinogen was applied. Thus the C:N values varied from 0.5 to 0.66; the ratio of vacate-oxygen to N from 1.3 to 2.0. The numerical values for the same animal were essentially similar.

OXIDATION-REDUCTION POTENTIALS OF THE BLOOD

As the oxidation-reduction potentials of the blood may, under certain conditions as shown by Oiwin (37), serve as an index of the state of oxidation processes in the organism, I made measurements of these potentials in an investigation undertaken in collaboration with M. Zalesskaya. The determinations were made with a syringe electrode of bright platinum according to the electrometric method of Kawetzky and Oiwin (18). The blood was drawn by syringe from the heart of the mouse. We used 60 mice, of which 20 were normal and 40 were painted with benzpyrene. We found that in mice painted with benzpyrene during a period of 3½ months the oxidation-reduction potential values varied on the whole within normal limits; namely, from 177 to 198 mv. Lower values were observed in only a few instances.

Mice with carcinoma induced by benzpyrene showed somewhat lower values of the oxidation-reduction potentials of the blood. This decrease occurred especially in the advanced stages of the process. Thus in normal mice the values of the potentials varied from 186 to 196 mv., while in the majority of mice with experimental carcinoma the values ranged from 172 to 184 mv., the difference between the mean values amounting to 7 mv. In the precancerous period, when papillomas developed, the values for the potential lay within the limits of normal variation. The data are presented in Fig. 2.

TABLE II: OXYGEN CONSUMPTION (VALUES FOR QO_2) BY SLICES OF LIVER FROM MICE WITH BENZPYRENE-INDUCED PAPILLOMAS AND CARCINOMAS AND FROM CONTROLS

Painted with benzene	Skin papillomas	Skin carcinomas
0.673	0.661	0.491 *
0.602	0.647	0.462
0.591	....	0.390
0.558	....	0.381
0.506	....	0.277
0.493	....	0.139
0.480	....	....
0.375	....	....
Averages:		
0.534	....	0.329

* Carcinoma in initial stage.

OXIDATION PROCESSES IN TISSUE SLICES

N. Ivashentsova studied the oxygen consumption by slices of liver and brain from mice which had been painted with benzpyrene. The determinations were made with Fenn's (13) apparatus, using either rabbit or horse serum as the medium for the tissue slices. Each experiment extended over a period of two hours. The control consisted of measurements of oxygen consumption by slices of liver and brain from mice painted with benzene alone. The data obtained for the liver are plotted in Fig. 3.

The data given in Fig. 3 show that there were no significant changes in oxygen consumption by slices of liver from mice painted on the skin with benzpyrene during a period of 3½ months, up to the appearance of carcinomas. The majority of the values obtained lie within the limits of the variations found in the controls, and the values were lower than normal only in a small number of experiments. Similar findings were obtained for the brain.

In Ivashentsova's study the oxygen consumption in slices of liver obtained from mice with carcinomas induced by benzpyrene was markedly reduced as compared with the normal. The data are presented in Table II.

It may be seen from Table II that in 7 out of the 8 control experiments QO_2 is above 0.480, whereas in all instances in which carcinoma was present, with the exception of the initial stages, the value of QO_2 was below 0.480. On the average the level of this coefficient was 39 per cent lower than normal. Two mice with papillomas had coefficients corresponding to the higher level of values in the controls.

Local Effect of Carcinogenic Hydrocarbons on Oxygen Consumption of Tissues

According to our findings, carcinogenic hydrocarbons, in the doses sufficient to induce the development of carcinoma of the skin in mice, did not have any appreciable effect upon the oxidation processes of the organism as a whole. Changes occurred only after the carcinomas appeared. On the other hand might not the carcinogen affect tissue respiration at the site of its application?

The investigation of this question was undertaken by our collaborator, N. Ivashentsova, who conducted experiments *in vitro* and *in vivo* to determine the effect of carcinogenic hydrocarbons upon oxygen consumption by tissues. A study was made first of the oxygen consumption by sections of liver and brain and by thin slices of the skin from the ears of mice, placed in serum containing a saturated solution of 1,2,5,6-dibenzanthracene (concentration about 4 mgm. per cent). Fenn's method was used. Under these conditions the respiration of tissues exposed to dibenzanthracene did not differ from that of the controls. The data obtained for the liver are given in Fig. 4.

These findings differ from those of Pourbaix (40-42) according to whom the addition of dibenzanthracene or benzpyrene to the medium causes a drop in the oxygen consumption of liver and brain. In her experiments, however, the decrease occurred only under certain conditions and was slight.

It may be objected that in these experiments of Ivashentsova there was not sufficient time for the penetration of the hydrocarbon into the cell and that the period of exposure of the tissue to the hydrocarbon was too short. Ivashentsova, therefore, studied the local effect on oxygen consumption in tissues painted with benzpyrene over a prolonged period. For this purpose ears of mice, which consist essentially of a double layer of skin separated by a thin layer of cartilage, were painted with benzpyrene dissolved in benzene for a period of 3½ months. Ears were amputated at various times during this period and their oxygen consumption was studied *in vitro*. The results of these tests showed that the oxygen consumption by tissues of the ears of mice subjected for periods of 1 to 3½ months to the action of benzpyrene did not differ from that of the controls. The data are presented in Fig. 5.

Glycolysis in tissue slices was not studied in our investigations.

In Pourbaix's (40-42) experiments, sections of liver and brain placed in an aqueous colloidal suspension of benzpyrene showed an increase in aerobic glycolysis in some instances, while anaerobic glycolysis remained unaffected. According to de Gaetani (8) benzpyrene is unable markedly to increase tissue glycolysis *in vitro*. Magat, Lebenson, and Wolkenson (30) on applying benzpyrene in small doses found no change in the consumption of sugar by fibroblasts in cultures.

Protein Metabolism

It has been our opinion that the primary changes in malignant cells consist in changes in the principal carrier of vital processes; namely, the protein component of protoplasm. For this reason we regarded as momentous Kögl's (20, 21) discovery of the stereochemical peculiarities of proteins of malignant cells.

Applying Kögl's method we analyzed a number of tumors induced by carcinogens. The first results of the incomplete investigation of A. Braun and N. Schmidt corroborate Kögl's findings with regard to glutamic acid. The data, presented in Table III, show that the proteins in the tumors produced by the carcinogens used were partially racemized.

DESCRIPTION OF FIGURES 1 TO 5

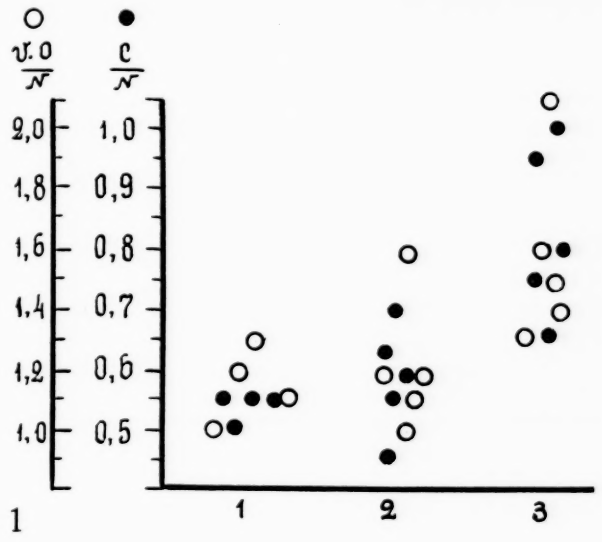
Fig. 1.—Data from experiments with benzpyrene painting. Urinary coefficients for $\frac{C}{N}$ and $\frac{\text{vacate-O}}{N}$ in mice. 1, normal mice; 2, mice with papillomas; 3, mice with carcinomas.

Fig. 2.—Graph showing the values for the oxydation-reduction potentials of the blood in 1, normal mice, and 2, mice with carcinomas induced by benzpyrene.

Fig. 3.—Oxygen consumption (in cu. mm. per mgm. of fresh tissue per hour) by slices of liver from mice which had been painted on the skin with benzpyrene dissolved in benzene and by similar tissue from control mice painted with benzene. Each experiment indicated by a circle, solid dot, or half-solid dot. The mean values are shown by horizontal lines. Fenn's method.

Fig. 4.—Oxygen consumption by slices of liver placed in serum saturated with benzpyrene or dibenzanthracene. On the ordinate are indicated the values of QO_2 in cu. mm. per 1 mgm. fresh tissue per hour with 1, normal serum, 2, serum containing benzpyrene, and 3, serum containing dibenzanthracene.

Fig. 5.—Oxygen consumption by ears of mice painted with benzpyrene dissolved in benzene during periods of 1 to 3½ months. The controls were painted with benzene alone.



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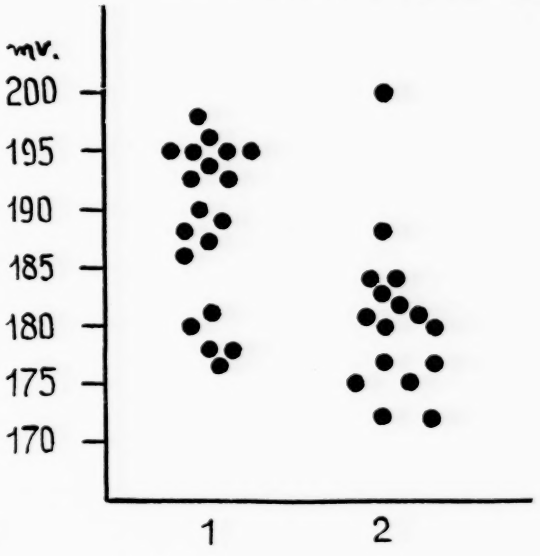

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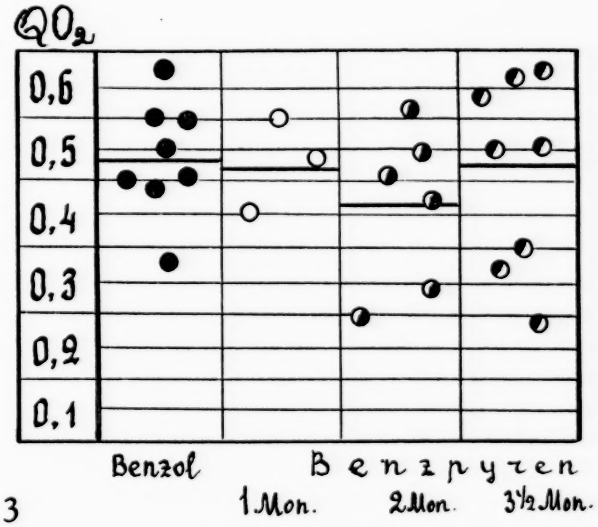

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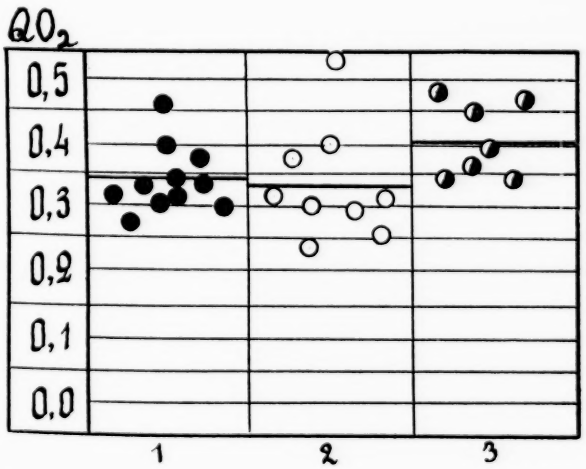

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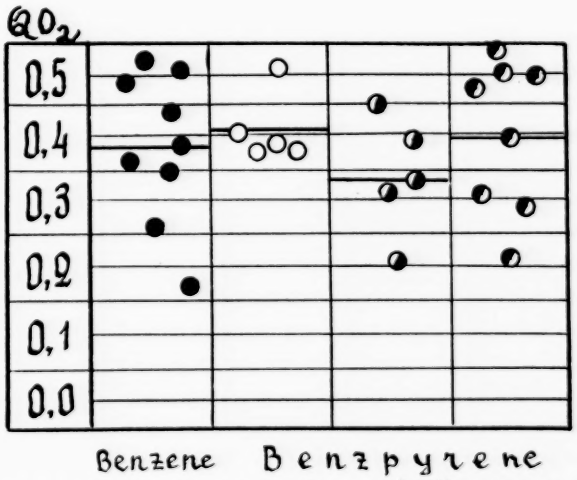


5

FIGS. 1 TO 5

Effect of Carcinogenic Hydrocarbons on Tissue Cultures

It is generally recognized that the problem of the pathogenesis of tumors would be greatly simplified if it were possible *in vitro* to change normal cells into malignant cells. Attempts have been made to bring about this transformation in tissue cultures, but the results obtained thus far have not solved the problem.

Evidence obtained from experiments with tissue cultures shows that the carcinogenic hydrocarbons are not growth-accelerating substances in the usual meaning of this term. This point has been brought out by the work of Larionow, Ivashentsova, and Tchertkova (26), Earle and Voegtlin (11, 12), Timofejevsky and Benevolenskaya (48, 49), and Katchka. It still remains to be discovered whether carcinogenic substances change normal cells to tumor cells by a direct and immediate action, or whether this effect is accomplished by means of an intermediate link. The positive results obtained with cultures of chick tissues exposed to tar and arsenic by Fisher (15), Laser (27), and by des Ligneris (9, 10) with dibenzanthracene appear to be doubtful because of the possibility of infection of the material with the virus of chicken sarcoma.

Table III: The Amount and Optical Characteristics of Glutamic Acid from Tumors Induced with Carcinogens and from Normal Tissues

Tissue	Glutamic acid: Amount in mgm.	Glutamic acid: $[\alpha]_D^{(\circ)}$	Glutamic acid: Percentage d-form
1. Transplantable dibenzanthracene mouse sarcoma	650	26.8	7.8
2. Transplantable methylcholanthrene rat sarcoma	115	23.5	13.12
3. Transplantable o-amidoazotoluol liver carcinoma of mouse	107	18.3	21.4
4. Normal mouse liver	350	31.1	0.96
5. Normal rat muscle	276	30.7	1.6

During the past three years in conjunction with my collaborators, M. Tchertkova and A. Samokhvalova (26), I have sought to obtain tumors in tissue cultures with benzpyrene and dibenzanthracene. After numerous failures we finally succeeded in obtaining a peculiar phenomenon in cultures of mouse fibroblasts. For explantation we used skeletal muscle of the hind leg of newborn mice. The cultures were grown in flasks. The first cells which grew were the cells of striped muscle and fibroblasts. In the course of reinoculations and transfers, the muscle cells disappeared and fibroblasts alone continued to grow. A mixture of chicken and rat plasma was used for the solid portion of the medium and diluted chicken embryo extract for the liquid medium. Fine aqueous suspensions of the hydrocarbons were prepared by precipitation from acetone solution according to Boyland's method. The hydrocarbons in this form were introduced into the cultures at the time of passages before coagulation of the plasma of the solid medium had occurred. The longest periods of cultivation were 1½, 2, 4, 4½, and 6½ months, respectively, in 5 series of experiments.

The special feature of these cultures was the appearance of secondary growth centers in the zone of proliferation (Fig. 6). The new daughter culture consisted of cells differing from those of the mother culture. These cells proliferated more rapidly, as a rule, than the elements of the original culture and soon surrounded the initial mass. Possessing greater mobility, these new cells became isolated from each other to some extent at the periphery of their zone of proliferation. The new cells differed also morphologically from the cells in the intitial growth. In most instances they did not form the radial strands characteristic of fibroblasts but were irregularly distributed. Some of the cells in some of the secondary centers of growth were distinguished by large size and peculiarity of form. They accumulated lipoid substances, often in large amounts, and appeared to differ biochemically from the cells of the primary growth. The characteristics of these cells are shown in Fig. 7.

Even small numbers of cells of some of the secondary growth centers were capable of giving rise to new cultures when transplanted, whereas with normal fibroblasts large numbers of cells were required for successful passages.

Unfortunately, through a series of accidents, nearly all of the secondary growth centers were lost during passages. In one case only was the growth of cells from a secondary center maintained for 2½ months without further addition of the carcinogenic compound.

The periods of time between the first introduction of the carcinogenic substance into the culture and the appearance of secondary centers were: for dibenzanthracene, 14, 24, 36, and 57 days; for benzpyrene 12, 14, and in the last series, 6 days. The concentrations of dibenzanthracene employed in the medium of the cultures varied from 0.3 to 0.6 mgm. per cent. In one series of cultures during a period of 14 days the concentration of benzpyrene was 0.15 mgm. per cent, while in the 6 and 12 days' series the concentration of benzpyrene was 0.6 mgm. per cent.

Our data on the effects of reimplantation of these altered cells into mice are not sufficient to justify conclusions. It is impossible, therefore, for us to say whether or not these cells from secondary growth centers are malignant.

As similar phenomena were observed in tissue cultures by Magat and Lebenson (28-30)), Timofejevsky and Benevolenskaya (48, 49), and Earle and Voegtlin (11, 12), it may be surmised with considerable prob-

ability that carcinogenic substances may act directly upon cells in the animal organism.

Effect of Nervous System on Incidence of Tumors

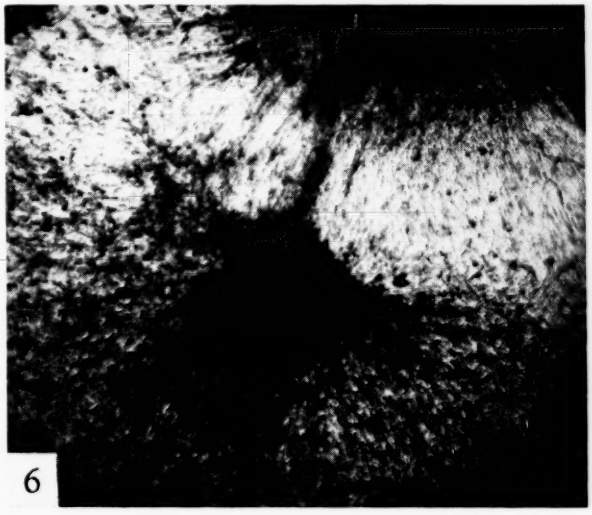
The probability of a direct action of carcinogenic substances upon normal cells, rendering them malignant, does not exclude the possibility that in this process other pathogenetic mechanisms may operate through intermediate links. The nervous system, for example, may play no part or a most important one.

Experiments were undertaken by L. Notik (36) to determine whether the nervous system influences the origination of experimentally induced cancer. For this purpose 114 mice were used. Of these, 62 had the skin in the lumbosacral region painted with tar during a period of 6 months, and 52 had similar areas of the skin painted with a 0.3 per cent solution of benzpyrene in benzene for 3½ months. In the precancerous period, at the time when papillomas appeared at the sites of painting, each series of animals was divided into two equal groups. One group served as control while in the animals of the other group the nervous system was "traumatized." In these mice the sciatic nerve was transected and its central end was treated with formalin or croton oil. Some of these animals developed trophic lesions of the joints of the foot on the side operated upon.

In mice subjected to traumatization of the nervous system in this manner papillomas which had developed following the application of the carcinogenic hydrocarbon regressed more frequently than in the controls and the incidence of carcinomas was less than it was among the controls. The data are presented in Table IV.

Table IV: Data on Regression of Papillomas and Incidence of Carcinomas in Mice Subjected to Traumatization of Nervous System

Carcinogen used	Period in months after painting	Regression of papillomas		Incidence of carcinomas	
		Control	Experimental	Control	Experimental
Tar	3	2	19	18	8
Benzpyrene	1½	1	17	13	2
Total		3	36	31	10



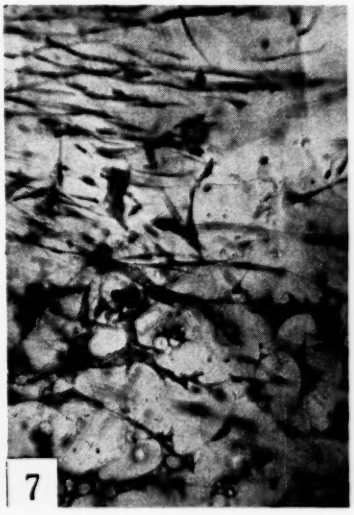


Fig. 6.—Low power photomicrograph of living tissue culture showing a secondary growth center. The initial mass of growth of mouse fibroblasts is shown at the top and below it the new center of growth formed on the 58th day of the action of dibenzanthracene.

Fig. 7.—Photomicrograph at higher magnification showing in the upper right region the normal fibroblasts of the mother culture and in the lower region the large, irregular, lipoid-containing, highly differentiated cells of the secondary growth center produced by dibenzanthracene. Formalin fixation; stained with hematoxylin according to Carazzi's method.

The results of these experiments showed that a pathological process evoked in the nervous system may influence the incidence or origination of cancer induced by carcinogenic hydrocarbons. Traumatization of the nervous system during the precancerous period inhibited the development of carcinomas. In the course of subsequent observations of the benzpyrene series evidence was obtained that this inhibitory nervous influence gradually abated with the subsidence of the pathological process in the nervous system. Carcinomas finally appeared, but at a considerably later period.

In another series of investigations, L. Notik applied the same method of traumatization of the nervous system much earlier, as soon as the benzpyrene painting was started. The lesions of the nervous system were thus produced before the onset of the pre-

cancerous period. A reverse effect was produced. Carcinomas appeared earlier than in mice of the control group. The results of these experiments are presented in Fig. 8.

The fact that an injury of the nervous system may have a different effect on the development of a pathological process according to the time of its application has been noted frequently by Speransky (46) and his collaborators. Although we cannot as yet say in what way the process of origination of experimental carcinoma is influenced by traumatization of the nervous system, the facts brought out by these experiments must be considered in the light of Speransky's theory that in the pathogenesis of pathological processes there is always a "nervous component."

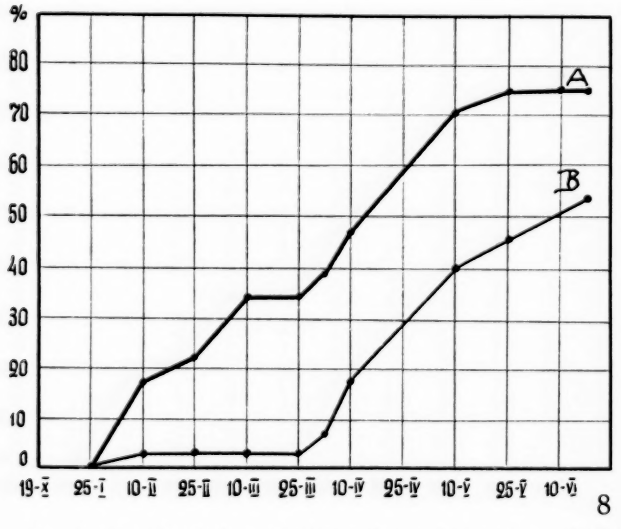


FIG. 8.—Graph of data of observations of the effect of the nervous system on the incidence of carcinomas induced by painting the skin of mice with benzpyrene. Heavy line (A): percentage of carcinomas among surviving mice in the experimental series; lighter line (B): percentage of carcinomas in controls.

DISCUSSION

Although comments have been made on the results of some of the experiments reported in this communication, more extended discussion of certain aspects of the problem is appropriate.

The biochemical data on oxidation processes and oxidation-reduction potentials of the blood correspond with the results of my investigations of the morphology of the endocrine glands in mice in which carcinoma of the skin was induced by painting with benzpyrene (24). Previous to the appearance of carcinoma no obvious morphological alterations could be detected in any of the endocrine glands. In particular the thyroid gland, which is one of the regulators of the oxidation processes in the organism, showed no morphological changes. On the other hand, I found that, after the appearance of carcinoma induced by applications of tar or benzpyrene, changes could be detected in the thyroid, parathyroid, thymus, suprarenal cortex, hypophysis, and ovary. A decrease in the function of the thyroid was especially significant in view of the important relation of this gland to processes of oxidation in the organism.

The mechanism of the development of the observed changes in metabolism requires special study. It may be that metabolism is affected by intoxication of the body by the products of destruction of portions of the tumors and by products of bacterial action in infected tumors.

The conception that carcinogenic agents primarily produce changes in the cellular metabolism in the organism, particularly in the respiratory processes of various organs, and that this is an indispensable condition for the development of tumors, was based in part on experiments with coal tar and arsenic. It is well known, however, that tar contains many toxic substances in addition to carcinogenic hydrocarbons and that arsenic is likewise a toxic substance. Our experiments with pure carcinogenic hydrocarbons indicate that this conception needs to be revised.

While coinciding with Neumann's (34, 35) data on the decrease in oxygen consumption by the liver when a tumor is present, our findings differ from his in respect to the respiration of the cells of internal organs previous to the development of tumors produced by carcinogenic hydrocarbons. Neumann obtained a slight drop in oxygen consumption and a rise in anaerobic glycolysis in liver and kidney previous to the appearance of sarcomas produced by carcinogenic hydrocarbons. Neumann, however, injected these substances in solution in sunflower oil. His own experiments showed that the injection of sunflower oil alone produced an almost identical decrease in oxygen consumption and increase in glycolysis in liver and kidney.

The results of our experiments furnish ground for the inference that in the case of the local action on tissues by carcinogenic hydrocarbons, under conditions that lead to the appearance of tumors, these substances are barely, if at all, able to affect oxygen consumption and carbohydrate metabolism of tissues. This does not mean, however, that under no conditions can changes in oxygen consumption and glycolysis occur. Different methods of tumor production or different carcinogenic compounds might perhaps have other effects. Thus, in the experiments of Hayashi and Tomita (16), on the application of o-aminoazotoluol, changes in oxygen consumption in the liver could be detected previous to the appearance of carcinomas. The questions are: to what degree are these changes characteristic of the action of carcinogenic agents; to what extent is their appear-

ance necessary; and are they essential for the process of transformation of normal cells into malignant cells? Our own data lead us to infer that a modification of oxygen consumption and glycolysis, which since Warburg's investigations have been considered characteristic of malignant cells, is less an immediate effect of the action of carcinogenic substances than a secondary phenomenon following upon some other primary changes in the cells, suffered while these cells undergo a transformation into malignant cells. It is to be noted in this connection that the recent work of Boyland (5, 6), Berenblum (4), and others has raised doubts as to the specificity of the changes in carbohydrate metabolism of tumor cells.

Summary and Conclusions

General systemic effects of carcinogenic hydrocarbons (benzpyrene) applied to the skin of mice were investigated by determinations of the ratios of partially oxidized substances excreted in the urine, the oxidation-reduction potentials of the blood, and the oxygen consumption of slices of organs. Local effects upon oxidation processes were studied in tissue slices and in skin to which the hydrocarbons had been applied. The oxidation processes were not disturbed during the precancerous period of papilloma formation, but supervened in a secondary manner after the appearance of carcinomas.

Incomplete investigations of proteins of induced tumors indicated that they contained an abnormally large proportion of d-glutamic acid.

The inclusion of the carcinogenic hydrocarbons, benzpyrene and dibenzanthracene, in the medium of tissue cultures of mouse fibroblasts produced changes in the morphological, biochemical, and proliferative characteristics of the cells. The data were not sufficient to indicate whether these cells had been transformed into malignant cells.

Traumatization of the nervous system by section of the sciatic nerve and treatment of the central end with formalin or croton oil affected the incidence of induced carcinoma in mice. An accelerating or inhibiting effect was dependent upon the time in the experimental cycle at which the lesions of the nervous system were produced.

The following conclusions are suggested:

1. Carcinogenic hydrocarbons applied to the skin of mice do not affect the oxidation processes during the precancerous period of papilloma formation.

2. Changes in the oxidation processes of the organism of some organs, and of tissues occurring in connection with carcinoma induced by carcinogenic hydrocarbons, are of a secondary nature.

3. The primary change caused by carcinogenic hydrocarbons may be an alteration of protein metabolism.

4. Carcinogenic hydrocarbons produce changes in cells by action directly upon the cells.

5. In the organism the nervous system seems to function as an intermediary link in the production of carcinomas induced by carcinogenic hydrocarbons.

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Artificial Benignancy of Neoplasm

VI. Observations on the Oxidative Behavior of Tumors, Artificially Benign Tumors, and Homologous Normal Tissues*†

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This communication reports a preliminary attempt to apply a chemical determination of cytochrome system activity to the routine classification of human and animal tissues. Such a measurement would be supplementary to routine histological findings that are not always interpreted alike by two or more competent pathologists. Over a decade ago the school of Warburg (23) attempted to adapt variations or analyses of the Pasteur reaction to such an application without great practical success. Nevertheless, recent developments in the field of enzymes concerned with oxidation justify a renewed effort in this direction. Accordingly, it is the purpose of this report to describe quantitative differences in oxidizing capacity between tumors, malignant and "artificially benign," and their normal homologues. The term "benignancy" is used here in a very general, gross sense to imply slow, localized growth without any connotation as to morbid histology.

In making such comparative measurements, it was remembered that one deficiency of the studies of glycolysis in the past decade or two has been the lack of suitable controls. In general almost any sort of malignant tissue has been compared with nearly every variety of normal organ. There are advantages in contrasting cancers with tissues as different as possible from them; but the results must not be interpreted as attributable to malignancy *per se*. If one wishes to limit the number of possible variables, obviously it is highly important to use pairs of tissues; *i.e.*, normal and malignant, of homologous histological types. For example, in this study malignant hepatoma has been compared with the liver of the animal host, or rhabdomyosarcoma with skeletal muscle from the animal host. Likewise implanted sarcoma 180 was compared with granulation tissue produced by a foreign body in the same animal. In this fashion one could assume that the normal and malignant cells being compared had been derived from the same embryonal source and had lived in the same *milieu interne*. Any differences observed between the normal and malignant cells would therefore have a greater likelihood of reflecting "malignancy" *per se*.

The purpose of the present experiments was to observe the oxidative behavior of such paired tissues in the presence of succinate or paraphenylenediamine. These substrates were selected in order to test the gross effectiveness of the succinic dehydrase and cytochrome systems in intact cells of the paired tissues. Anaerobic observations with methylene blue are in progress to study specifically the activity of succinic dehydrase *per se*, but the present report is not primarily concerned with this feature.

Material and Experimental Methods

The tissues used in these studies were the following:

Connective tissue.—For the malignant form, Crocker mouse sarcoma 180 was transplanted into Bagg albino A mice; for the normal control, kieselguhr granuloma was induced in the same mice by the method of Neuhaus (15) by injecting subcutaneously a suspension of silicious earth. The "artificially benign" sarcomas were produced by immunization, as described in a preceding publication (13).

Liver tissue.—For the malignant form, hepatoma was induced in strain C albino mice by the subcutaneous injection of 2-amino-5-azotoluene, as described by Andervont (1). We are indebted to Dr. Andervont for the parent tumor from which transplants were made. The normal control tissue was obtained both from the identical host and from untreated animals.

Muscle tissue.—For the malignant form, methylcholanthrene in lard was injected subcutaneously into C3H agouti mice, as described by Stewart (20). From tumors so produced transplants were made into animals of the same strain. The normal control tissue

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was diaphragm, removed whole, both from the identical host and from untreated animals.

Experimental procedure.—The experimental procedure was the manometric estimation of tissue metabolism described in previous studies (13). Estimations of oxygen consumption and glycolysis were performed manometrically in the single vessel, constant-volume apparatus of Warburg (23). The medium used in determining oxygen consumption was mammalian Ringer's solution containing 0.2 per cent glucose and maintained at pH 7.4 with phosphate buffer. To this medium was added succinate or paraphenylenediamine prepared as follows: A stock solution of 0.2 M succinic acid was neutralized to pH 7.4 and a sufficient quantity taken to bring the final concentration of succinate to 0.02 M. The paraphenylenediamine was freshly prepared in a concentration of 2 per cent, and enough of this solution was added to the Warburg vessel to provide 1 mgm. of paraphenylenediamine per cc. of the final medium.

In the case of the latter substrate, a special control vessel was regularly used in addition to the usual thermobarometric control. By this means a blank was obtained which corrected for autoxidation.

At the conclusion of each manometric determination, the tissue slices were removed and dried to

Table I: Comparative Metabolism of Normal and Malignant Homologous Tissues

Experiment No.	1 Q_{O_2}	2 $Q^{O_2}_{CO_2}$	3 $Q^{N_2}_{CO_2}$	$\frac{3-2}{3}\times 100$	Experiment No.	1 Q_{O_2}	2 $Q^{O_2}_{CO_2}$	3 $Q^{N_2}_{CO_2}$	$\frac{3-2}{3}\times 100$
LIVER TISSUE									
	Normal liver (mouse)					Liver tumor (mouse)			
438	10.3	2.2	1.6	— 37	. . .	10.4	2.6	9.5	73
462	10.1	2.8	1.4	—100	. . .	11.0	1.3	10.5	88
466	13.5	5.0	3.0	— 66	. . .	18.2	7.9	21.1	63
470	13.1	2.7	2.4	— 13	. . .	12.7	5.3	16.0	64
471	11.5	4.0	3.5	— 14	. . .	10.8	6.6	19.2	66
474	13.2	3.3	1.0	—230	. . .	13.7	8.1	20.9	61
475	8.2	4.7	1.2	—291	. . .	9.8	4.1	6.2	34
477	7.6	2.5	0.7	—265	. . .	7.9	1.0	15.2	93
481	8.3	4.6	1.6	—187	. . .	7.4	0.9	11.6	92
504	11.2	4.8	4.6	— 4	. . .	13.0	2.7	24.4	89
530	12.4	7.4	8.5	+ 13	. . .	10.1	12.5	25.0	50
531	12.6	4.3	2.7	— 59	. . .	12.3	9.1	20.9	52
532	13.9	5.7	5.8	+ 2	. . .	12.4	8.3	20.9	60
535	10.7	6.5	5.7	— 14	. . .	10.3	6.1	19.8	69
536	10.9	6.9	4.7	— 49	. . .	10.5	6.7	23.0	71
Av.	11.2	4.5	3.2	— 88	Av.	11.3	5.5	18.3	63
MUSCLE TISSUE									
	Diaphragm muscle (mouse)					Muscle tumor (mouse)			
619	9.0	3.2	9.1	65	507	6.1	7.1	16.4	53
623	5.8	0.0	5.7	100	510	7.4	7.1	16.6	57
	7.1	1.8	8.0	78	514	12.9	10.8	30.3	64
	6.7	1.0	5.8	83	521	9.3	7.5	20.7	64
626	3.9	2.1	5.7	63	522A	10.0	7.1	22.7	69
	5.6	0.2	5.9	97	522B	9.7	8.5	30.1	72
	4.3	2.6	7.1	63	526	9.2	11.1	23.7	53
627	5.2	0.5	6.2	92	618A	6.7	5.7	11.3	50
	4.4	1.9	6.4	70	618B	8.2	6.5	11.3	42
	2.7	0.6	8.8	93	619	7.8	5.6	12.4	55
Av.	5.5	1.4	6.9	81	Av.	8.7	7.8	19.6	58
CONNECTIVE TISSUE									
	Kieselguhr granuloma (rat) *					Spindle cell sarcoma (rat) *			
Av.	3.8	8.7	11.9	25	Av.	5.9	22.2	29.4	23
						Sarcoma 180 (mouse) †			
					Av.	7.0	14.4	24.7	40

* Data taken from Neuhaus (15).
† Data taken from Muus, Craig, and Salter (3).

constant weight. Therefore the weights listed in the accompanying tables are lower than the corresponding values which might have been found if the same tissues had been dried and weighed without being tested. This point has been discussed by Field, Belding, and Martin (10).

Preliminary Metabolic Observations

First of all, the triad of paired tissues named above was compared from the standpoint of oxygen consumption and of glycolysis in the presence of glucose. The values for (*1*) Q_{O_2}, (*2*) $Q^{O_2}_{CO_2}$, (*3*) $Q^{N_2}_{CO_2}$ and (*4*) the percental Pasteur effect were formulated as described by Warburg (23) or Dean Burk (4) to indicate cu. mm. of gas per mgm. of "final" *dry* tissue per hour. Typical results are assembled in Table I, which includes data of Neuhaus (15) on sarcoma and granulation tissue in the rat. In general, these data show the increased anaerobic, and even aerobic, glycolysis described by Warburg as a characteristic property of many malignant tissues.

Other data do not clearly demonstrate a quantitative relationship between "malignancy" and glycolysis. In the series of liver tumors, the extent of anaerobic glycolysis was compared with the corresponding mitotic activity, by the procedure of Brues and Salter (3). As shown in Table II, there was little correlation between mitotic activity and glycolysis. To be sure, this correlation is seldom claimed and is therefore of doubtful expectancy; but the data show concisely that one could not hope for aid in classifying a given tissue on the basis of such data. The coefficient of correlation was only 0.15 if no points were excluded. An attempt to grade the tumors for malignancy by routine pathological examination also failed to show any qualitative correlation with the rate of glycolysis. In brief, the present experience with this triad of homologously paired tissues is typical of the bewildering mass of data stimulated by Warburg's original concept—although hardly by Warburg himself.

Furthermore, a comparison of oxygen consumption, as summarized in Table I, failed to show any consistent differences between normal and malignant tissues. In the case of muscle and of connective tissue, but not of liver, there was a higher average Q_{O_2} in the malignant form.

In passing, it might also be noted that liver commonly shows the negative percental Pasteur effect, illustrated in Table I, although scarcely any other tissue does so. In other words, $Q^{CO_2}_{O_2}$ is greater than $Q^{N_2}_{CO_2}$, and the difference would have been even more marked if in the calculations the true R.Q. of 0.6 to 0.7 had been used instead of the arbitrarily assumed value of 1.0.

Observations on the Effect of Succinate

When the homologous tissues were supplied with an adequate amount of succinate in addition to glucose, it became obvious that the normal tissues responded by increased oxygen consumption much more than did the malignant tissues. The data are shown in Table III. In brief, succinate increased the utilization of oxygen by normal cells much more than it did that of tumor cells. These results are summarized in Table IV and Fig. 1.

It will be noted from the data that this conclusion holds for all three pairs of tissue only when the results are computed on a percental basis. On an absolute basis, it was true for liver and muscle, but not for the connective tissues; *i.e.,* granulation versus sarcoma. Both of these last named tissues responded by approximately the same increment in the value for Q_{O_2}; *i.e.,* by about 2 units. In this respect the granulation tissue differed from the natural "normal" tissues and, in absolute terms, was more like the malignant form. In general, however, percental increments are more interesting than absolute values; and because the original Q_{O_2} value for granulation was low, its small incremental response to succinate was the more impressive. On the other hand, the original oxygen consumption of sarcoma was high, so that the incremental response appeared relatively insignificant.

Because of the clear response to succinate, extensive observations with paraphenylenediamine were not made because of its toxic effect noticeable after 60 minutes of contact with the tissues. This length of time is quite adequate for the present purpose; and,

Table II: Comparison Between Rate of Anaerobic Glycolysis and Frequency of Mitotic Figures in Hepatoma

Experiment No.	$Q^{N_2}_{CO_2}$	Mitotic figures per 1,000 cells *
438	9.5	3.9
462	10.5	26.1
470	16.0	7.9
471	19.2	10.3
474	20.9	6.6
475	6.2	5.7
477	15.2	9.6
481	8.4	5.6
529	18.1	5.3
530	25.0	10.6
531	20.9	5.8
532	20.9	7.7
533	15.9	4.9
534	11.3	8.8
536	23.0	8.7
Average	14.8	8.0

Coefficient of correlation = 0.147; *i.e.*, poor.
* In each case 1,000 cells were counted by the procedure described by Brues and Salter (3).

TABLE III: EFFECT OF SUCCINATE ON OXYGEN UPTAKE OF PAIRED HOMOLOGOUS TISSUE, NORMAL AND MALIGNANT

Normal				Malignant			
Experiment No.	Q_{O_2} with succinate		Per cent change	Experiment No.	Q_{O_2} with succinate		Per cent change
	0	0.02 M			0	0.02 M	
NORMAL LIVER COMPARED WITH HEPATOMA							
477	7.6	14.1	86	477	7.9	6.5	−18
481	8.3	24.3	193	481	7.4	13.5	82
504	11.2	28.5	154	504	13.0	15.6	20
530	12.4	32.8	164	530	10.2	11.3	11
531	12.6	29.9	137	531	12.3	11.4	− 7
532	13.9	25.9	86	532	12.4	12.6	2
535	10.7	29.5	176	535	10.3	14.1	37
536	10.9	24.5	125	536	10.5	13.1	25
578	11.3	34.1	201	578	14.0	12.9	− 8
590	11.4	19.8	74	590	9.2	12.6	37
	9.9	25.1	154		10.1	11.1	10
	9.4	20.8	120		9.4	10.9	16
595	8.5	18.3	115	595	9.4	12.8	36
Mean	10.6	25.2	137	Mean	10.5	12.2	17
NORMAL MUSCLE (DIAPHRAGM) COMPARED WITH RHABDOMYOSARCOMA							
524	7.1	...	...	507	6.1	10.1	66
...	8.1	26.8	231	510	7.4	11.1	50
525	3.9	28.0	618	514	12.9	24.9	93
526	6.1	17.0	179	521	9.3	13.8	48
527	...	23.3	...	522	10.1	10.0	1
528	8.3	23.7	186		9.7	14.5	49
	8.6	20.2	135	526	9.2	7.6	17
	8.0	21.9	174	540	9.6	13.4	40
540	2.9	21.9	656		8.2	13.5	65
569	11.1	23.7	113	567	7.3	13.4	84
	12.0	27.0	125	569	7.5	9.5	27
595	3.4	15.9	368		11.0	13.7	25
	5.5	19.6	256	584	9.8	12.3	26
	5.1	11.4	124		9.2	12.8	39
599	7.0	17.9	156		7.1	10.3	45
	6.7	17.9	167	586	7.0	9.1	30
	7.7	18.5	140		9.0	10.3	14
	...	...	...		6.4	8.7	36
	...	...	...	567	8.9	13.4	51
Mean	7.0	20.9	242	Mean	8.7	12.2	41
GRANULATION TISSUE COMPARED WITH SARCOMA 180 AND "BENIGN" SARCOMA 180							
551	0.40	1.70	325		*Sarcoma 180*		
	1.10	5.30	381	551	6.5	10.1	55
	...	2.30	...		9.7	11.9	23
553	1.47	4.10	179		8.6	10.5	22
	2.09	4.22	102	553	8.0	9.8	23
	0.74	3.41	361		8.8	10.6	21
554	1.95	3.65	86	554	8.2	10.8	32
575	2.80	5.00	79	575	8.8	8.5	− 3
	1.67	4.35	160		7.1	8.3	17
580	1.65	3.78	129	580	9.0	13.6	51
582	1.05	2.57	145	582	7.2	9.1	26
	1.26	4.74	276		10.3	9.3	−10
	1.73	3.96	129		5.7	9.7	70
595	1.91	4.73	148	Mean	8.2	10.2	27
Mean	1.52	4.14	192				
					"Benign" sarcoma 180		
				557	7.2	8.5	18
					7.4	9.1	23
				606	4.1	6.2	51
					4.3	6.1	42
					5.5	7.6	38
				610	6.9	8.8	28
					4.2	7.7	83
				662	8.6	10.1	17
					8.8	11.3	29
					7.2	10.1	42
				Mean	6.4	8.6	37

moreover, Rhoads (17) and his collaborators have noted a differential toxic effect of paraphenylenediamine which becomes manifest after this interval. A few such observations were made to be certain that normal tissue responded well to paraphenylenediamine and malignant tissue did not. In these experiments the rate of oxygen consumption with this substance was approximately that with succinate, a fact which indicated that succinic dehydrase was not a limiting factor in these normal tissues; *e.g.,* liver or muscle. Later in this report will be found data obtained with mammary tissue in which failure of succinic dehydrase complicated the situation. It must be remembered, however, that the present communication is concerned chiefly with the gross over-all activity of the entire cytochrome system.

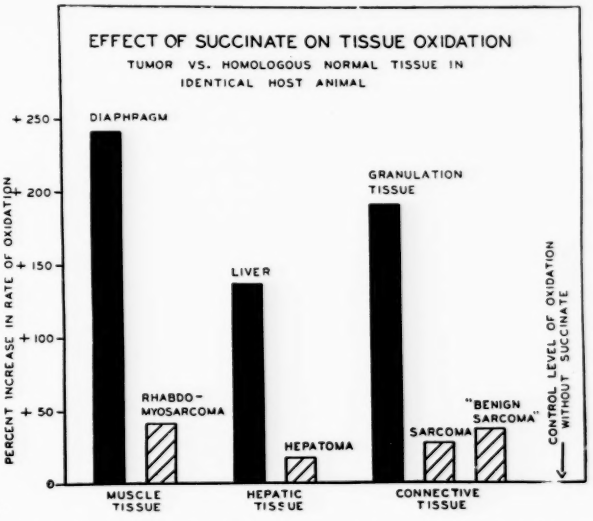

FIG. 1.—Three pairs of homologous tissues, normal and malignant, derived from the identical host, were treated with succinate and the resulting increase in oxygen consumption measured. The normal tissues, represented by solid block bars, responded more strikingly than did their respective malignant homologues.

ARTIFICIAL BENIGNANCY COMPARED WITH NATURAL BENIGNANCY

In previous publications from this laboratory the behavior of "artificially benign" sarcomas has been described in several respects. For example, growth rate and mortality were shown to be less than for control sarcomas. The rate of mitotic division was lower, and the rate of oxygen consumption of the benign tumors was less than for malignant, control sarcomas. These findings raised the following questions: (a) In the artificially benign sarcomas had the fundamental malignant process been reversed or (b) was the benign character of the neoplasms merely a reflection of an environment unfavorable to the rapid growth of implanted neoplasms? These two alternatives have been referred to in a widely publicized report (16) as involving, respectively, (a) the "causal" genesis and (b) the "formal" genesis of neoplastic disturbance. The issue is obscured further by the concept of "humoral resistance" which has been considered as a separate phenomenon by some investigators.

In order to test which of these alternatives was involved in the artificial benignancy previously studied, a chemical comparison of "benign" and ma-

TABLE IV: COMPARISON OF SUCCINATE OXIDIZING CAPACITY IN THREE HOMOLOGOUS PAIRS OF NORMAL AND MALIGNANT TISSUES

	Liver		Muscle		Connective tissue		
	Normal	Hepatoma	Normal	Rhabdomyosarcoma	Normal (granulation tissue)	Sarcoma 180	"Benign" sarcoma 180
A. No. of determinations							
Control	13	13	16	16	13	14	10
Succinate	13	13	19	19	12	12	10
B. Q_{O_2} control	10.6	10.5	7.0	8.7	1.52	8.2	6.4
C. Q_{O_2} in 0.02 M succinate	25.2	12.2	20.9	12.2	4.1	10.2	8.6
D. Standard deviation							
Control	±1.8	±3.8	±2.5	±1.7	±0.6	±1.3	±1.8
Succinate	±5.8	±2.2	±4.5	±3.7	±1.3	±1.1	±1.7
E. Increase in Q_{O_2} due to succinate (C-B)	14.6	1.7	13.9	3.5	2.6	2.0	2.2
F. Incremental increase, per cent of control $\left[100 \times \frac{E}{B}\right]$	+137%	+17%	+242%	+41%	+192%	+27%	+37%
G. Probable coincidence, P-value * (Succinate versus control)	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001	0.01
H. Ratio of per cent incremental increase in normal tissue to per cent incremental increase in malignant tissue	$\frac{137}{17} = 8.1$		$\frac{242}{41} = 5.9$		$\frac{192}{27} = 7.1$		$\frac{192}{37} = 5.2$
I. Probable coincidence, P-value * (Normal versus malignant)	<0.001		<0.001		<0.001		0.29 †

* Calculated according to the method described by R. A. Fisher.
† Compared with control sarcoma 180.

lignant sarcomatous tissue was made. To this end, highly malignant and artificially "benign" sarcomas 180 were derived from the *same* tumor by implantation into inbred mice of the Bagg albino A strain. These tumors were compared with granulation tissue produced in the same strain of mice. The object of the observations was to determine whether the utilization of succinate would show that, in contrast to the homologous malignant sarcoma, the "artificially benign" tumor behaved like granulation tissue.

In our previous chemical study (13) of malignant versus "artificially benign" sarcoma, no difference was found either in anaerobic glycolysis or in aerobic glycolysis. The only difference noted was a 30 per cent depression of oxygen consumption in the "benign" tissue. The effect of succinate likewise failed to disclose a difference. As shown in Table IV, succinate produced a marked percental increase in oxygen consumption by nonmalignant granulation tissue, but caused only a small elevation with the two sarcomas 180; *i.e.,* the rapidly growing and the slowly growing forms. In all three cases, the increment was small in absolute terms.

This result, therefore, suggests that in the "artificially benign" sarcoma the "formal" factor remains latent. In other words, the benign effect is environmental. The chemical evidence agrees with biological evidence already reported; namely, that when "artificially benign" tumors are inoculated into fresh hosts, the resulting daughter tumors are highly malignant. Thus tumor growth may be inhibited successfully by humoral changes even though its *potential* malignancy remains unaltered.

Discussion

The observations just described indicate that the malignant tissues used are deficient either in succinic dehydrase or in the cytochrome-oxidase system. Collateral experiments with paraphenylenediamine have shown that this substrate yields the same experimental result as succinate. Accordingly, the determining deficiency is in the cytochrome system, even though the succinic dehydrase may be low too. Stotz (21) has pointed out that the deficiency may reside either in the cytochrome or in its oxidase, and his direct measurements in tissue extracts indicated that both were low in the tumor cells which he studied.

Studies by Rosenthal (18) in surviving liver tissue and by Elliott and Greig (9) in homogenized animal tissues indicate that the oxidation of succinate often reflects the activity of the cytochrome system, and that this system seems to be much less active in tumors than in many normal tissues. Elsewhere the present report describes observations on breast tissue in which normal lactating breast failed to respond to succinate, but did respond to paraphenylenediamine. Accordingly, the use of both substrates is desirable to exclude simply a deficiency in succinic dehydrase. To this end, furthermore, the anaerobic use of methylene blue as hydrogen acceptor is convenient.

It should be observed that in the calculation of the data the response to succinate or paraphenylenediamine has been estimated as a percental increment above the respiration in glucose alone. This was done in fear that nonrespiring or poorly respiring elements might be interspersed throughout the tissue parenchyma. This procedure presumably minimized the effect of such stroma elements as might adulterate the parenchyma. The data do not concern cyanide-sensitive as contrasted with cyanide-insensitive respiration—a point studied by van Heyningen (22) and reviewed by Commoner (8).

Fortunately, necrotic tumors usually show values for respiration which differ but little from that for healthy tumor. Nevertheless, special emphasis has been laid upon the technical necessity of adding successive slices of the tissue alternately to the control and paraphenylenediamine vessels, respectively. In this way the effect of necrosis and of stroma, which ought to adulterate the respiring tissue parenchyma, tends to be cancelled out. The two absolute values for Q_{O_2} in each homologous pair have, therefore, not been emphasized in this report, and the percentage increase over the control value has been selected as the significant value. Obviously this device may conceivably fail when either the stroma or necrotic area far outweighs visible parenchyma. For this reason the biochemical results must be evaluated in the light of the general histological architecture of the tissue under consideration. The use of adjacent tissue slices possibly also has the advantage over the direct determination of enzymic activity in extracts; namely, that partial correction is made for nonviable tissue.

Other authors have compared homologous tissues, normal and malignant, by the study of glycolysis or respiration. Thus Neuhaus (15) studied sarcoma and granulation tissue in the rat. Similarly, as summarized by Kinosita (12), Nakatani, Nakano, and Ohara studied primary hepatoma (induced by butter yellow) in comparison with normal liver, and found a progressive increase in glycolysis as the malignant state was approached. More recently Berenblum, Chain, and Heatley (2) compared the Shope rabbit papilloma with skin epithelium, but found no difference in glycolysis in the potentially malignant stage. In view of the generally accepted failure of glycolysis to provide an objective criterion of malignancy in individual samples of malignant tissue, however, the simple method of testing for cytochrome system activity should be exploited further. Possibly other

oxidative enzyme systems might be studied similarly, because Kensler, Sugiura, and Rhoads (11) have pointed out that livers bearing tumors induced by butter yellow are deficient in flavin.

APPLICATION OF THE PROCEDURE TO TEST CASES

In the preceding section a metabolic difference between certain tumors and their normal homologues has been described. This difference involved the activity of the succinate or cytochrome system. It was suggested that such a procedure might be useful in distinguishing malignant from nonmalignant tissues. It is the purpose of the following pages to describe a few such trial experiments. The procedure used has been described in the foregoing part. The tissues employed were (*1*) mammary tumor, murine and human, (*2*) human leukocytes, (*3*) human skin tumor, and (*4*) rabbit papilloma, induced by the Shope virus.

TABLE V: OXIDATIVE BEHAVIOR OF MAMMARY TUMORS, MURINE AND HUMAN

Material	Exp. No.	Succinate			Paraphenylenediamine		
		Control	Q_{O_2}	Per cent increase	Control	Q_{O_2}	Per cent increase
Normal mouse lactating breast	...	1.9	2.6	+37	...	6.3	+231
		4.3	...	...	...	4.8	12
		3.8	...	...	...	5.0	32
		0.3	...	...	...	1.5	362
		4.1	...	...	...	5.7	39
		0.4	...	...	...	1.5	298
Murine mammary scirrhous carcinoma (transplanted tumor I)	631	8.8	11.4	+30	13.8	12.7	— 8
		8.6	9.7	13	11.5	14.4	+25
		6.8	11.0	62	9.1	11.4	25
		10.3	10.5	2	10.0	11.4	14
		8.7	9.1	5	10.6	13.2	25
		8.3	9.7	17	8.5	13.9	64
	632	7.7	9.9	29	11.0	13.6	24
		7.5	10.0	33	8.8	13.8	57
		6.6	9.9	50	11.5	10.1	—12
		8.2	9.1	11	10.4	13.8	+33
		8.3	9.3	12	...	...	...
		7.5	10.5	40	...	...	...
		11.5	10.5	—10	...	...	...
		10.4	12.4	+19	...	...	...
Murine mammary adenocarcinoma (spontaneous tumor II)	1	6.8	...	...	...	7.9	16
	2	6.4	...	...	...	7.4	15
	3	4.8	...	...	...	5.9	23
	4	5.1	...	...	...	7.0	37
	5	4.6	...	...	...	5.5	19
	6	8.4	11.6	+38	...	10.6	+27
Human breast tumors (carcinomas)	X	1.5	15.3	...	...	14.0	+830
	Y	1.68	...	...	...	2.89	+87
	Z	1.31	1.99	+52	...	1.31	0

OBSERVATIONS ON MAMMARY TUMORS

Murine breast.—Two tumors of the mammary gland in mice were studied. The first of these arose spontaneously in the Dobrovolskaia-Zavadskaia strain of albino mice and was transplanted into inbred mice of the same strain. This tumor resembled a scirrhous mammary carcinoma. It is designated in Table V as murine breast tumor I. The second murine breast tumor was derived from Roscoe B. Jackson Laboratory strain A mice exhibiting a high incidence of spontaneous tumor. It is designated in the table as murine breast tumor II. In order to eliminate the factor of transplantation, the observations in the case of the latter tumor were made directly on primary tumors arising spontaneously. In Table V are collected observations on these two murine tumors compared with normal lactating murine breast. These results are especially interesting with regard to the effect of succinate. This substrate failed to disclose a marked difference between the normal lactating breast and the two malignant tissues, but when paraphenylenediamine was employed, the characteristic failure of the malignant tissue to respond was demonstrated. Presumably the normal breast was poor in succinic dehydrase but showed a high cytochrome system activity. It should be explained, however, that it is technically not easy to obtain satisfactory sections of mouse breast tissue, even during lactation. Consequently not much emphasis is laid upon such data in this report. The percental increment for the normal tissue was

over ten times that of the tumor in those instances where the response was more than minimal, but the results were erratic.

Human breast.—In Table V also are shown results obtained with three human breast tumors excised at operation. These are designated as X, Y, and Z, respectively. It is interesting that the results ranged from a very marked response, such as was found with normal mouse tissue, to practically no response. A brief synopsis of the microscopic findings follows.

Murine mammary adenocarcinoma, tumor II. An adenocarcinoma showing secondary lumina, not well differentiated, with many mitoses. The tumor contains large medullary masses; definitely but not markedly malignant.

Human breast tumor X. A carcinoma, possibly adenocarcinoma. The tumor is poorly differentiated; probably slowly infiltrating. There is some trace of lumen formation. Not many mitoses. Cells and nuclei are fairly uniform.

Human breast tumor Y. An adenoscirrhous carcinoma. Very poorly differentiated and clearly infiltrating surrounding tissue. There is marked variation in size of the nuclei and mitoses are frequent. The tumor is rapidly growing.

Human breast tumor Z. Chronic mastitis with comedo carcinoma. The tumor is growing chiefly in the ducts but has escaped. There is a moderate number of mitoses. Low-grade malignancy is indicated in slowly growing tumor.

Human leukocytes and lymph node.—Because of the association of leukemia with malignant disease, the procedure was applied to human leukocytes in 2 cases of lymphatic leukemia, 3 cases of myelogenous leukemia, and 1 case of lymphosarcoma. The method of dealing with the leukocytes was as follows:

The drawn venous blood was prevented from clotting with heparin (1 mgm. per cc.). The leukocytes were separated by centrifugation in specially designed centrifuge tubes with a constricted neck located between upper and lower reservoir spaces. The buffy layer so obtained was removed by aspiration into a capillary pipette and transferred to a Warburg's manometric respirometer containing Ringer phosphate glucose medium, reinforced with heparin to a concentration of 0.5 mgm. per cc. The results were expressed in terms of the dry weight of the leukocytes, which were collected after the experiment by centrifugation from the Ringer's medium.

The results, shown in Table VI, revealed no essential difference between the normal and leukemic massed white cells. Both groups showed little or no response to succinate, but a marked response to paraphenylenediamine. In this connection, further studies

TABLE VI: HUMAN LEUKOCYTES

Experiment No.	Description of case: Diagnosis	Description of case: W.B.C. (cu. mm.)	Q_{O_2} in Ringer's phosphate: Without addition	With glucose	With succinate	With paraphenylenediamine
		CONTROL BLOOD				
637	Normal	.....	...	4.8	2.1	7.7
638	Normal	.....	...	4.9	3.9	7.2
640	Normal	.....	...	3.3	2.4	10.0
641	Normal	.....	...	2.7	3.0	7.5
646	Normal	.....	5.1	..	2.9	24.1
647	Normal	.....	3.9	3.7	3.5	11.4
Average				3.9	3.0	11.3
		LEUKEMIC BLOOD				
634	Lymphatic	33,000	...	4.8	3.9	...
635	Mycloge nous	400,000	...	3.0	2.8	...
643	Lymphatic	100,000	...	6.3	2.5	13.5
644	Mycloge nous	200,000	4.6	2.4	3.4	11.1
645	Lymphatic	30,000	5.5	4.9	5.5	8.0
648	Mycloge nous	400,000	4.6	3.0	4.4	10.2
649	Monocytic	170,000	...	4.4	5.6	12.3
Average				4.1	4.0	11.0
651 *	Lymphosarcoma	.....	23.7	18.0	14.7	14.1
651	Lymphosarcoma	.....	...	13.1	...	...
652	Lymphosarcoma	.....	7.1	4.8	3.6	12.0
653 *	Lymphosarcoma	.....	...	5.9	...	11.5
653	Lymphosarcoma	.....	...	5.3	...	12.0
		HUMAN LYMPH NODE				
...	Hodgkin's disease	.....	...	2.22	3.1	4.7

* Sternal bone marrow blood.

on the succinic dehydrase of leukocytes would be interesting.

Human skin.—In Table VII are shown results obtained from the skin of 3 patients: (*1*) normal skin, (*2*) a gumma of the corium excised from the leg, and (*3*) a nodule excised from the thigh of a patient suffering from a disease respectively described as mycosis fungoides or lymphosarcoma by two competent pathologists. The results indicate that the normal skin and the gummatous tissue responded remarkably to the presence of paraphenylenediamine, whereas epithelioma from the lip failed to respond. Of special interest is the the biopsy of "mycosis fungoides." This tissue had been diagnosed Hodgkin's disease and lymphosarcoma, respectively, by two other well-known pathologists. The patient's condition became rapidly worse. It is interesting that the test with paraphenylenediamine showed that this tissue behaved like a malignant tissue by this method.

In addition, data are also given for an epithelioma of the lip.

Rabbit papilloma induced by the Shope virus.—The Shope virus was introduced into the scratched skin of 3 rabbits. Throughout the course of 3 months thereafter, biopsies were taken of the normal skin and of the papillomatous lesion. At first the papillomatous tissue responded well to the presence of both succinate and paraphenylenediamine. Between the 7th and the 10th week, however, this property was lost, indicating from the standpoint of the biochemical test that the tissue had begun to behave like a malignant tissue. At this time no metastases were detectable, although there was some slight evidence of ulceration. Histological sections were prepared at the same time that these determinations were made, for comparison. Four illustrative descriptions by Dr. John Houghton are given below, two at 6 and 7 weeks, when the papilloma still responded to succinate and paraphenylenediamine; and two later determinations at 10 and 14 weeks, when the biochemical response was absent.

Specimen 4/3/41 (6 weeks). A "prepapillomatous" lesion of the skin, showing hypertrophic epithelium with but little hyperkeratosis. The basement layer is intact. There is a moderate number of mitotic figures in the deeper layers. The cell nuclei are uniform in size, without notable hyperchromatism or giant nuclei. Diagnosis: Early papilloma without marked deviations from normal skin.

TABLE VII: SKIN AND SKIN TUMORS, HUMAN AND RABBIT

Description	Q_{O_2} in Ringer's phosphate glucose				
	Without addition	In 0.2 M succinate		In 0.2 per cent paraphenylenediamine	
		Q_{O_2}	Per cent change	Q_{O_2}	Per cent change
HUMAN					
Normal skin	0.12	...	...	0.40	+200
Gumma of corium	0.16	...	...	0.49	+210
Epithelioma (lip)	0.72	...	...	0.51	−29
Mycosis fungoides (? lymphosarcoma)	5.2	...	...	6.7	+29
RABBIT *					
Normal skin					
Preliminary biopsy	0.17	...	...	1.32	+790
At 15 weeks	0.87	...	...	1.89	+118
At 25 weeks	1.04	...	...	3.84	+270
Shope virus papilloma					
At 6 weeks	1.07	2.38	+222	3.38	+316
At 7 weeks	0.28	1.05	+382	1.22	+440
At 10 weeks	3.04	3.89	+28	3.05	<+1
At 14 weeks	2.59	2.70	+4	2.93	+14
	3.79	3.46	−9	3.27	−14

* Inoculated with Shope virus by Dr. F. Sargent Cheever.

Specimen 4/8/41 (7 weeks). Not much different from the preceding section, although marked hyperkeratosis is present.

Specimen 5/2/41 (10 weeks). An evident papillomatous lesion, with marked hyperkeratosis and hypertrophied epithelium. The basement layer is intact. There is a marked papillary superstructure. The nuclei are fairly uniform in size, without notable hyperchromatism or giant nuclei. In the deeper layers there is a moderate number of mitoses. The degree of hyperplasia is greater than in the preceding sections, and there is more crowding of the nuclei seen in each microscopic field. Diagnosis: Papilloma, not carcinoma.

Specimen 5/26/41 (14 weeks). The biopsy shows a papillomatous lesion with much folding of the epithelium, which is well differentiated. Intercellular bridges are present deep down in the epithelium. There is a very thick cornified layer. Mitoses are numerous, but there is no hyperchromatism. The nuclei show slight variation in size and shape. There is no pearl formation and the basement layer is intact. Diagnosis: Papilloma, not carcinoma.

Discussion

The work of Rous (19) suggests that papillomas of this type in domestic rabbits usually undergo malignant change ultimately. From this standpoint it is suggestive that at the 10th and 14th week, when the biochemical result already indicated a malignant change, as shown in Table VII, the histological report was "papilloma, not cancer." The possibility must therefore be entertained that a characteristic enzymic change in tissue cells may herald malignant or premalignant transformation for a considerable time (weeks or months) before malignant changes can be recognized by microscopic examination.

Berenblum, Chain, and Heatley (2) studied "the metabolism of normal and neoplastic skin epithelium" in the domestic rabbit. These investigators measured the oxygen uptake, the aerobic and anaerobic glycolysis, and the R. Q. of normal skin epithelium and of Shope virus papilloma. They obtained values for normal epithelium which were almost identical with those for the papilloma and very similar to values cited in the literature for many squamous carcinomas. They concluded, therefore, that "aerobic glycolysis and a low R. Q. of a glycolyzing tissue are both normal physiological processes, and do not represent a pathological disturbance characteristic either of tumor growth or any other lesion." Unfortunately, it is not clear from the limited details given in their paper whether they were dealing with the Shope papilloma in its malignant or premalignant stage. It is therefore impossible to compare their data with our results included in this report.

The findings in human leukemia reported here are in accord with the observations of Burk, Sprince, Kabat, and Furth (7) on the metabolism of chicken tumors and leukoses. These investigators studied glycolysis and found that whereas the chicken tumors showed the marked alterations in glycolysis characteristic of many highly malignant tissues, as described by Burk (5), on the other hand the leukoses behaved quite like normal tissue; *e.g.,* spleen or bone marrow hyperplasia induced by acetylphenylhydrazine. Similarly Burk, Behrens, and Sugiura (6) studied the metabolism of butter yellow rat liver cancers. They found, in contrast to the report of Nakatani, Nakano, and Ohara (14), that just before the origin of obviously malignant tissue, an increase in anaerobic as well as aerobic glycolysis occurred. The data reported here for liver hepatoma similarly produced show a low cytochrome system activity in the final stage, but studies are not available at the moment on the actual transition from cirrhotic liver to hepatoma. The analogous observations, however, with Shope virus papilloma, indicate that the loss of cytochrome system activity definitely precedes the presence of histologically perceptible malignancy. Evidence that normal glycolysis may also be present has been described by Berenblum, Chain, and Heatley (2).

In general, these observations on decreased cytochrome system activity are consistent with the thesis advanced by Burk (5) that malignant change in cells is accompanied by the utilization of enzymic pathways not ordinarily called upon to bear the brunt of normal metabolism. The evidence seems sufficiently suggestive to warrant further application to human cases of malignant disease.

Summary and Conclusions

By the use of succinate and paraphenylenediamine it has been possible to test the activity of the cytochrome system in homologous normal and malignant tissues from the same host. Thin slices of surviving tissues were used. Connective tissue, liver, and muscle were studied in inbred mice, and in all three types the malignant but not the normal form showed low cytochrome system percental response. On the contrary, "artificially benign" sarcomas 180 in immunized hosts behaved as if malignant. A few instances are described in which the procedure has been applied to mammary tissue (human and murine), to human leukocytes in health and in leukemia, to a human lymph node, and to nodules of the human skin. The circulating leukocytes in human leukemia respond to paraphenylenediamine as do normal leukocytes. In rabbits, papillomas of the skin produced by the Shope virus have been studied at intervals during the transition between the benign and malignant form. In this last material, a loss of cytochrome system response occurred rather abruptly after several weeks, and before histological evidence of frank malignancy was present.

These findings have been compared with collateral histological sections. The preliminary results suggest that the biochemical method of studying animal and human tissue outlined in this paper might be a useful adjunct to routine morphological study.

The authors are greatly indebted to Doctor Dean Burk for his careful critical review of this report, and for many helpful suggestions.

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The Influence of Sex of Mice on Acquired Resistance to a Transplantable Sarcoma*

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Recently reported experiments of the author (7) have indicated that male mice are more susceptible to intradermal implantation of sarcoma S 37 than are females. First, it was noted that when small, carefully measured doses of tumor cell suspension were inoculated, the incidence of takes was higher in male mice than in females. Secondly, it was found that in male mice fewer tumors underwent spontaneous regression than in females. While the difference in the incidence of takes between males and females could easily be abolished by injecting the more concentrated tumor cell suspensions, the difference in the incidence of spontaneous regression of the cutaneous tumors was evident in all groups of experiments including those in which large doses of sarcoma were implanted intradermally.

The present experiments were designed to determine whether male and female mice in which tumors had regressed spontaneously were equally immune to reinoculation with the same tumor.

Experimental

Four hundred and twenty-seven males and 470 females were inoculated intradermally with cell suspensions of sarcoma S 37. The technic of preparing the tumor cell suspension and inoculating the mice has been described elsewhere (7). Sexually mature male and virgin female albino mice[1] 2 to 3 months of age and weighing 18 to 24 gm., were used throughout this study. As shown in Table I, cutaneous tumors were produced in 384 male mice and in 375 females. These tumors, histologically typical sarcomas, regressed spontaneously in 47 of the male animals (12 per cent) and in 161 of the females (43 per cent). Forty-two of the recovered males and 132 of the females were reinoculated either intradermally or intraperitoneally with 0.05 to 0.2 cc. of a 20 per cent suspension of sarcoma S 37. As controls for the malignancy of the tumor cell suspensions, normal male and female mice were inoculated with equal doses of the same tumor suspension at the same times as the recovered animals.

The results of these experiments are shown in the accompanying Table II.

According to these data, male mice were definitely less immune to either intradermal or intraperitoneal reinoculation with sarcoma S 37 than were females, 11 of 42 male animals developing tumors upon reinoculation as compared with 4 of 132 females. The control experiments with normal male and female mice show that the large doses of tumor cell suspensions used produced tumors in practically all of the inoculated animals of both sexes.

Discussion

It has been known since the work of Clowes and Baeslack (6) in 1905 that mice which recovered spontaneously from successful tumor implantation are in most instances resistant to a subsequent reinoculation with the same tumor. Systematic studies on this subject were facilitated by the observation of Besredka and Gross (2-5) that the incidence of regressing growths in transplantable tumors in mice is substantially increased by implanting small doses of the tumors intradermally instead of subcutaneously, as is the routine procedure. Thus they found that animals which recovered from tumors produced by intradermal inoculation were resistant to future reinoculation with the same tumor by any of the usual routes.

The observation of Besredka and Gross was confirmed by Andervont (1), who suggested at the same time that female mice which have recovered from tumors produced by intradermal inoculation are more resistant than males to reinoculation with the same tumor. This suggestion is substantiated by the results of experiments reported in Table II of this paper.

* This investigation was aided by a grant from Mrs. Francis F. Prentiss.

[1] The white mice used in this study were obtained from the colony of Mr. E. Schwing, at Harrison, Ohio. According to Mr. Schwing, this colony of mice was started some 35 years ago from a single pair of albino mice. Breeding by brother-to-sister mating has been continued since that time. These mice reach sexual maturity at 40 to 45 days of age. About 5 per cent of breeding females develop mammary tumors between 12 and 24 months of age.

TABLE I: INCIDENCE OF SPONTANEOUS REGRESSION OF TUMORS PRODUCED BY INTRADERMAL IMPLANTATION OF SARCOMA S 37 IN ADULT MALE AND FEMALE MICE

Tumor, per cent concentration	Tumor suspension, cc. injected	Sex	No. of mice inoculated	No. of mice developing tumors	No. of mice tumors regressed	Per cent spontaneous regression
1	0.02-0.03	M	18	5	2	40
		F	20	2	1	50
5	0.02-0.03	M	18	11	1	9
		F	18	3	1	33
5	0.02-0.03	M	17	16	2	12
		F	21	12	7	58
5	0.03-0.04	M	9	4	1	25
		F	14	6	5	83
10	0.02-0.03	M	20	12	1	8
		F	20	9	3	33
10	0.02-0.03	M	21	20	4	20
		F	17	17	9	53
10	0.03-0.04	M	14	14	3	21
		F	14	5	4	80
10	0.03-0.04	M	12	10	3	30
		F	17	9	5	55
15	0.03-0.04	M	18	17	3	18
		F	14	11	5	45
15	0.03-0.04	M	19	19	2	11
		F	18	18	3	17
20	0.02-0.03	M	20	20	2	10
		F	21	21	11	52
20	0.02-0.03	M	17	17	2	12
		F	21	20	13	65
20	0.02-0.03	M	9	9	1	11
		F	8	8	1	13
20	0.02-0.03	M	13	13	0	0
		F	16	15	6	40
20	0.02-0.03	M	21	21	2	10
		F	20	20	9	45
20	0.03-0.04	M	11	11	1	9
		F	15	15	6	40
20	0.03-0.04	M	11	11	1	9
		F	15	13	5	38
20	0.03-0.04	M	8	8	2	25
		F	8	8	6	75
20	0.03-0.04	M	2	2	0	0
		F	5	3	3	100
20	0.03-0.04	M	21	21	3	14
		F	20	20	10	50
20	0.03-0.04	M	10	5	0	0
		F	15	7	2	29
20	0.03-0.04	M	19	19	1	5
		F	20	20	8	40
20	0.05	M	10	10	1	10
		F	9	9	4	44
20	0.06	M	15	15	2	13
		F	15	15	3	20
20	0.06	M	13	13	1	8
		F	28	28	8	29
20	0.20	M	12	12	1	8
		F	12	12	5	42
25	0.10	M	18	18	2	11
		F	18	18	10	55
25	0.20	M	18	18	2	11
		F	18	18	5	28
30	0.10	M	13	13	1	8
		F	13	13	3	23
	Total	M	427	384	47	12
		F	470	375	161	43

In conclusion, it should be pointed out that there are now three lines of experimental evidence suggesting that adult male mice may be less resistant to tumor implantation than are adult females: first, the greater incidence of takes in males; second, the lower incidence of spontaneous regression of cutaneous tumors produced in males; and third, the lesser incidence of immunity in recovered males to reinoculation with the same tumor.

TABLE II: THE RESISTANCE OF RECOVERED MALE AND FEMALE MICE TO REINOCULATION WITH SARCOMA S 37

Experiment No.	Inoculation with 20 per cent suspension of sarcoma	Recovered * mice: Sex	No. inoculated	No. developing tumors	Per cent developing tumors	Normal control mice: Sex	No. inoculated	No. developing tumors	Per cent developing tumors
1.	0.06 cc. intradermal	M	9	5	..	M	15	15	..
		F	43	2 †	..	F	15	15	..
2.	0.05 cc. intradermal	M	7	2	..	M	10	10	..
		F	11	0	..	F	9	9	..
3.	0.20 cc. intraperitoneal	M	17	2	..	M	15	15	..
		F	54	1	..	F	15	15	..
4.	0.20 cc. intraperitoneal	M	9	2	..	M	20	19	..
		F	15	0	..	F	26	24	..
5.	0.15 cc. intraperitoneal	F	9	1	..	F	18	17	..
Summary:	all experiments	M	42	11	26	M	60	59	98
		F	132	4	3	F	83	80	96

* The term "recovered" is used to designate those animals in which an intradermal inoculation of tumor cell suspension was followed by formation of a tumor and subsequent spontaneous and complete disappearance of the neoplasm.
† Both tumors disappeared promptly.

SUMMARY

Male and female mice, in which tumors produced by intradermal inoculation of sarcoma S 37 disappeared spontaneously, were reinoculated intradermally or intraperitoneally with the same tumor. Practically all females were resistant to reinoculation, whereas one-fourth of the males developed tumors.

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A Comparison of the X-Zone of the Adrenal Cortex in Two Inbred Strains of Mice*

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Recent work by Woolley, Fekete, and Little (6, 8) has demonstrated that in certain inbred strains of mice striking changes in the adrenal cortex follow early castration. The female mice of the dilute brown (Dba) strain, castrated at birth, showed areas of nodular hyperplasia in the adrenal cortex, after one year involving and partly replacing all three zones of the cortex. These mice also showed marked development of the mammary glands. It was suggested that the action of the hyperplastic adrenals stimulated the development of the mammary glands. It was further shown that the adrenal hyperplasia occurred in two strains of mice with a high incidence of spontaneous mammary tumors (Dba and C3H) and was absent in the C57 black strain which has an exceedingly low incidence of spontaneous mammary carcinoma.

The differences between the castrated females of the Dba and C57 strains suggested the possibility that differences might be detected in the adrenals of intact females of the two strains. A comparison of the adrenal glands of two strains of mice varying in their incidence of mammary tumor has been made by Cramer and Horning (2). They attempted to demonstrate a relation between "brown degeneration" of the adrenal gland and the appearance of mammary tumors. This brown degeneration consisted of necrotic tissue in the peripheral part of the medulla. Recently Blaisdell, Gardner, and Strong (1) have investigated the problem. They concluded after a study of 11 different strains of mice that there was no consistent relation between frequency of brown degeneration and the potentiality of any strain to develop mammary cancer.

While studying the early stages of adrenal changes following castration, we noted a histological difference between a high tumor strain and a low tumor strain. The appearance and persistence of the x-zone of the adrenal in one strain of mice differed from those of the other strain.

The x-zone, as first described by Howard (4) and confirmed by Deanesley (3), is a transitory juxtamedullary element of the adrenal cortex of the mouse. The x-zone is present in both sexes before puberty but regresses rapidly in the male at this time. In the female it persists for a variable period and eventually regresses, often with widespread vacuolization. The original descriptions indicated that there might be considerable differences in the structure and relationships of the x-zone in different strains of mice. The Danforth strain used by Howard showed an x-zone which was considerably more prominent than that of most other strains and was accompanied by very extensive vacuolization in the process of regression. The x-zone of the adrenal of mice of this strain averages 55.2 per cent of the width of the cortex as compared to an average width of less than 15 per cent of the cortex in 10 mice of the Dba strain in females between 45 and 55 days old (5).

The object of this paper is to report a difference between the x-zone of the adrenal in the intact female and castrated mice of both sexes of the Dba and of the C57 black strains, and to describe the x-zone of the hybrid females of a cross between the two strains.

Materials and Methods

Two strains of inbred mice were used, the JAX Dba which has a high incidence of mammary tumors and the C57 black strain which has a very low incidence. Both strains have been extensively inbred and for the purposes of this work may be considered genetically uniform. Care was taken to keep the mice under as nearly identical conditions as possible. Factors which would affect the condition and structure of the x-zone; *i.e.* infection, starvation, pregnancy, etc., were guarded against.

In the castrated series, operations were performed at 1 day, 21 days, and 40 days of age. Mice castrated at 1 day were anesthetized by chilling. The older animals were anesthetized by intraperitoneal injection of nembutal. At the time the mice were autopsied the condition of the genital system was noted to determine whether the castration had been successful.

The left adrenal was used for histological examination. As soon as possible after the death of an animal tissues were fixed in Bouin's or Tellysnicky's fluid. The glands were imbedded in paraffin and serial sections were made. Adequate differentiation of the x-zone was obtained by routine staining with hematoxylin and eosin.

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Measurements were made of the cortex and x-zone with a calibrated ocular micrometer. Sections were selected for measurement which showed the maximum area of medulla. In this way a section near the center of the gland was used.

present at birth but appears between 10 to 20 days of age. In the males the x-zone parallels the development of the females until about 30 days. During the next 10 days a regression occurs in th x-zone of both strains so that by the 48th day the x-zone can no longer

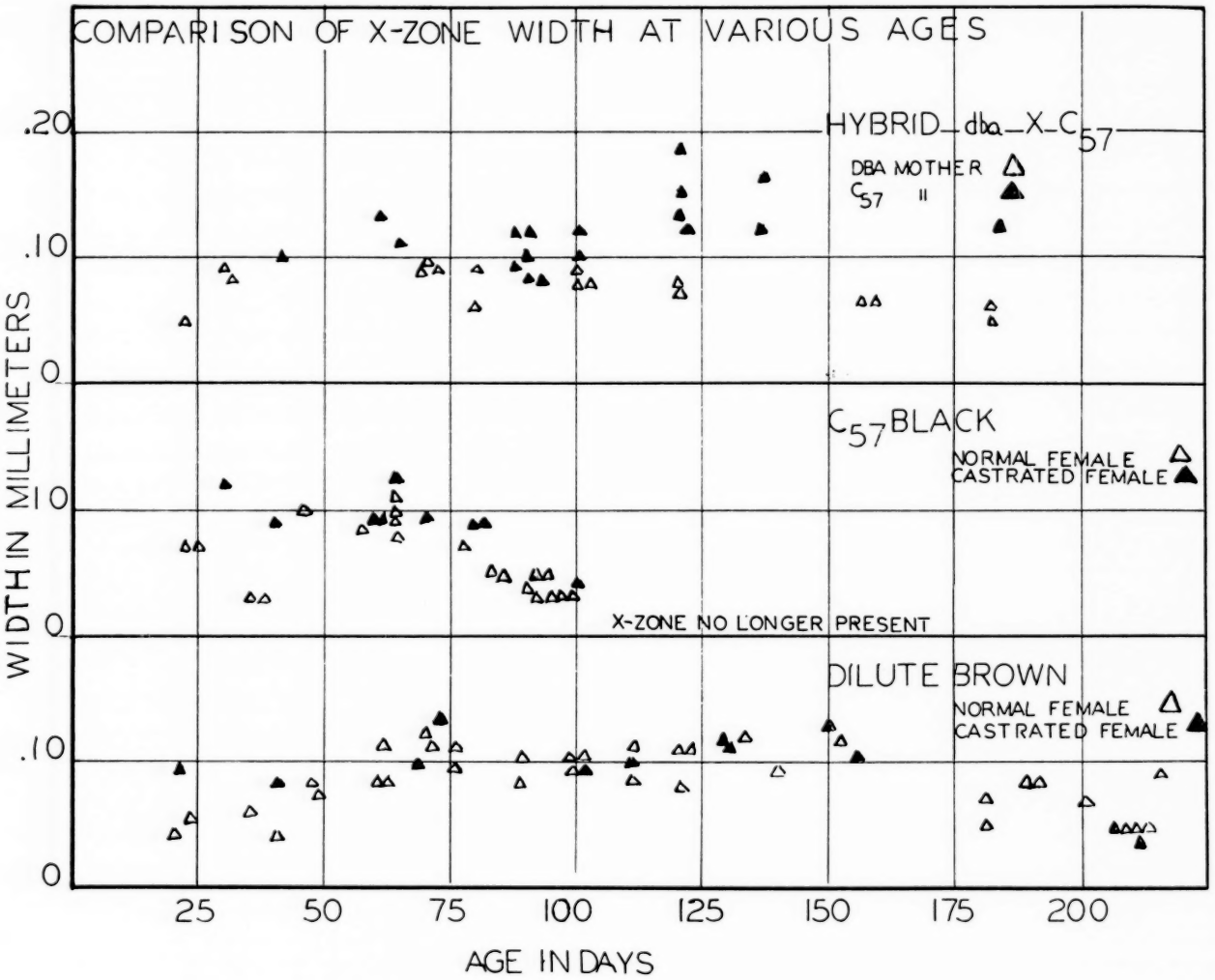

FIG. 1

OBSERVATIONS

Measurements of 204 adrenal cortices have been made of mice of the following types:

I. Dba:—virgin females, 37; males, 22; castrated females, 13; castrated males, 11.

II. C57 black:—virgin females, 38; males, 17; castrated females, 15; castrated males, 5.

III. Hybrid (Dba x C57 black):—Dba ♀ x C57♂, 17; C57 ♀ x Dba ♂, 21.

The adrenals of normal males of the Dba and C57 black strains of mice possess x-zones which are essentially similar to the strain described by Howard (4). As in the females of these two strains, no x-zone is be recognized. In both strains the process of regression is unaccompanied by lipoid degeneration such as is found in the Dba females and the females of the Danforth strain described by Howard. Following the regression of the x-zone the adrenal cortex of the male mouse has reached its permanent pattern and undergoes no marked change until senile changes occur.

The measurements of female mice have been compared graphically in Fig. 1. The linear thickness of the x-zone is plotted against the age of the mouse.

The development and regression of the x-zone in the Dba and C57 black strains of mice follow the general descriptions of Howard (4, 5) and Deanesley (3). In comparison to the variable appearance of the x-zones in random bred mice the x-zones in each

inbred strain presented a uniform and predictable appearance.

The most obvious difference between the x-zones of the two strains is the early reduction of the width of the x-zone of the virgin C57 black female beginning at about 70 days. At 100 days it can no longer be identified as a distinctive region of the cortex. The virgin female of the Dba strain possesses an x-zone which persists for over twice as long. The x-zone of this strain does not begin to show a terminal regression until 190 to 200 days and it may persist at 210 days or slightly longer.

The x-zones of the two strains also differ in the degree of vacuolization. The vacuolization which played such a large part in the degeneration of the x-zone of the Danforth strain of mice, described by Howard, is found in the Dba mice but to a lesser extent. In the C57 black strain vacuolization does not take place in the x-zones of the adrenals of the virgin female. The degeneration proceeds entirely by a regression and shrinking of the cells, unaccompanied by fatty degeneration.

Castration of either male or female before puberty resulted in the persistence of the x-zone. The histological appearance and duration of the persistence in each case resembled the normal virgin female of the strain. The adrenal changes following castration reported by Woolley, Fekete, and Little (6) began as localized nodules of cortical cells in the glomerular region, quite distinct from x-zone material.

An unusual anomaly was noted in one castrated male of the Dba strain. This animal was castrated at 40 days of age and killed when 130 days old. In addition to the expected finding of a definite x-zone still prominent at this age, the adrenal had an accessory gland associated with it which showed the same differentiation into x-zone and remaining cortical tissue as found in the main gland. This accessory adrenal was entirely devoid of medullary elements.

Virgin females which were hybrids of the two strains showed x-zones more closely akin to the Dba parent type. The x-zone was still intact at 180 days of age and the hybrids showed the vacuolization found in the Dba strain. It was found that females which had C57 mothers had on the average a larger x-zone than those with Dba mothers. Although the numbers are small it was found that between the ages of 90 to 180 days the former group of hybrids had about a 50 per cent larger x-zone.

Discussion

The function of the x-zone is as yet unknown and therefore the differences observed between these strains of mice cannot at present be given any definite physiological significance. The described differences in the x-zone of strains whose adrenal glands show such a different reaction to castration may well, however, be more than a chance coincidence.

Cramer and Horning (2) have stated that the x-zone is to be associated with the medulla. The occurrence of typical x-zone cells in an accessory gland entirely devoid of medullary elements is evidence that the two types of tissue may exist separately.

The use of genetically uniform strains of mice will help to throw light on the nature and function of the x-zone. To ascertain the effect of experimental procedures it will be of advantage to have strains of mice whose x-zone shows distinguishable and reproducible characteristics.

Summary

1. A difference between the x-zone of virgin females of the Dba and C57 black strains of mice is reported. The x-zone of the Dba mice persists for over 200 days and its regression is accompanied by vacuolization. The x-zone of the C57 black mice on the other hand undergoes complete regression by 100 days without vacuolization.

2. The x-zones of the male and female mice castrated before puberty resemble those of the virgin females of the same strain.

3. The x-zone of the hybrid of the Dba and the C57 black strains resembles the Dba parent. Hybrids having the C57 black mother seem to show a larger x-zone than those having a Dba mother.

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A Genetic Analysis of the Induction of Tumors by Methylcholanthrene

III. Local and Remote Induction of Carcinoma of the Mammary Gland*

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In 1939, Strong and Smith (21) reported on the local induction of carcinoma of the mammary gland in mice by methylcholanthrene. Ten female mice that were being used as breeders developed such tumors at or near the site where the carcinogen had been injected subcutaneously (1 mgm. dissolved in 0.1 cc. sesame oil and injected at 60 days of life). Eight of these mammary tumors were in NH strain mice; 2 were in mice of the JK strain. It was noteworthy that such mammary carcinomas had been induced in only 2 out of 7 distinct inbred strains of mice used in the experiment. It was further significant that these 2 strains (NH and JK) were genetically related and were both characterized by a very low incidence of spontaneous tumors of the mammary gland.

Burdette (5) obtained 1 adenocarcinoma of the mammary gland in a CBAN breeder female mouse by the biweekly painting of a 1 per cent solution of methylcholanthrene dissolved in benzene to the skin of the back for 139 days. Kirschbaum (10) obtained 5 similar tumors in F strain female mice. These tumors occurred in 3 of 5 mice which had been injected intravenously with a suspension of methylcholanthrene in horse serum and 2 occurred in mice injected intraperitoneally with the same carcinogen and vehicle. Mice of the F strain are similarly very resistant to the occurrence of spontaneous tumors of the mammary gland. Mice of the CBAN strain show an intermediate degree of susceptibility. It has recently been reported by Bonser (2) that the injection of relatively large amounts of methylcholanthrene (2 mgm. dissolved in lard given subcutaneously in the right flank in 2 doses at an interval of 5 weeks) will produce tumors including adenocarcinomas of the mammary gland in mice. In a later report in which the histology of these tumors was described, Bonser and Orr (3, 4) stated that 23 of the 160 tumors induced in the above series were adenocarcinomas of the mammary gland. Nine of these were unassociated with other types of neoplasias and all occurred in female mice at the site of injection of the carcinogen. It is not stated whether or not the female mice were being used for breeding.

The occurrence of mammary tumors in rats subsequent to the implantation of paraffin pellets containing variable amounts of methylcholanthrene has been reported by Dunning, Curtis, and Eisen (8). The changes in the mammary tissue adjacent to the wax cysts were described and the major effect appeared to be upon the duct epithelium. Examination of a representative number of the nodular areas which occurred immediately proximal to the implanted material revealed that from 15 to 20 per cent of the masses contained mammary tissue. Among these, from 12 to 45 per cent contained areas of squamous epithelium. In from 5 to 30 per cent there was evidence in the mammary ducts of the transition of columnar into squamous epithelium. Areas of metaplastic squamous epithelium were more frequent in nodules from female hosts but were not confined to them. The earliest changes were observed in females, 9 to 30 days after injection. These consisted of the partial transformation of the epithelium of the mammary ducts and the formation of squamous epithelium in hyperplastic mammary ducts. In the total number of tumors examined, 159 or 17 per cent contained areas of breast tissue, of which approximately one-half showed hyperplastic changes, and about 30 per cent of which contained squamous epithelium. In only 5 per cent, however, was the relationship between the mammary ducts and the presence of squamous epithelium uninvolved with tumor growth. These investigators stated further "the mechanism of the action of methylcholanthrene on breast epithelium is unknown but a direct irritative effect appears probable. The reaction is not comparable with the effect of the estrogens."

Perry and Ginzton (14), using unpedigreed albino stock mice (origin unknown), reported carcinomas of the breast in spayed female mice painted with a 0.3 per cent benzene solution of 1,2,5,6-dibenzanthracene and theelin. They did not obtain mammary carcinomas by the carcinogen alone in normal female mice, but did report six obtained in normal mice with the carcinogen and theelin. They conclude that the "incidence of carcinoma of the skin is chronologically related to the development of carcinomas of the breast."

Materials and Methods

One of us (16, 18) has been making a survey of the specific types of tumors and an analysis of the frequency distribution induced in mice of the NH descent by 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil and injected subcutaneously at 60 days of life. Ultimately it is intended to analyze this material genetically, in order to ascertain whether or not genetic factors are involved in the origin of these specific types of induced tumors.

This communication presents observations on 800 mice of the NH strain, of which 384 were females and 416 were males. The mice were weaned at 30 days of age and placed in reserve until 30 days later,

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at which time, 60th day of life, 1 mgm. of methylcholanthrene dissolved in 0.10 cc. of sesame oil was injected subcutaneously on the right side. The sexes were not separated and the ensuing young were discarded within 24 hours post-partum (forced breeding).

Female mice of the NH descent have, up to the present time, never developed spontaneous tumors of the mammary gland (either in the virgin state or under forced or normal breeding). The present series of 800 methylcholanthrene-injected mice includes *all* mice in lineal descent. Consequently variability as brought about by selection following hybridization cannot be a factor in influencing frequency distribution and specific types of tumors induced in this present experiment.

Results

The data obtained with reference to mammary gland changes (hyperplasia, metaplasia, and neoplasia) are given in Table I. Forty-five mice showed definite evidence of carcinoma of the mammary gland, 40 of which were the usual adenomatous type found in mice (Figs. 1 and 2) and 5 were of the scirrhous type, less frequently found in cases of spontaneous mammary carcinoma. Eleven of the carcinomas of the adenomatous type showed squamous metaplasia (Figs. 3, 4, and 5). Of the 45 cases of carcinoma of the mammary gland, 21 occurred unaccompanied by any other type of tumor, whereas 24 were associated in varying degrees of anatomical intimacy with other types of tumors[1] as follows: 12 with spindle cell sarcoma, 8 with carcinoma of the skin, 1 with primary carcinoma of the lung and rhabdomyosarcoma, and 3 with spindle cell sarcoma and carcinoma of skin.

In addition to these 45 definite carcinomas of the mammary gland, two other changes in mammary tissue were also found. These were (a) 9 cases of nonmalignant hyperplasia and (b) 11 cases of squamous metaplasia of mammary tumors (all in tumors of the adenomatous type). These alterations occurred in mice showing tumors other than those of mammary origin as follows: 7 cases of mammary hyperplasia associated with spindle cell sarcoma (Fig. 6); 2 cases of hyperplasia with spindle cell sarcoma and carcinoma of the skin; 3 cases of squamous metaplasia of mammary tumors occurring along with carcinoma of the skin; and 8 cases of squamous metaplasia of neoplastic mammary tissue unassociated with any other type of tumor (Figs. 3 and 4).

In all instances squamous metaplasia was associated with cyst-like formations, marked keratinization, and extensive inflammation. In certain areas the mastitis was in a definitely suppurative state; in others, small accumulations of polymorphonuclear leukocytes were the only evidence of inflammatory processes. The general picture, however, indicated that in all of the metaplastic tumors an extensive mastitis had been present for a considerable time.

All mammary changes occurred only in female mice. The frequency distribution of mammary tumors is compared to those for other types of tumors in these 800 NH mice in Fig. 7.

Table I: Data on Tumors and Lesions of Mammary Gland Observed in 384 Female NH Mice

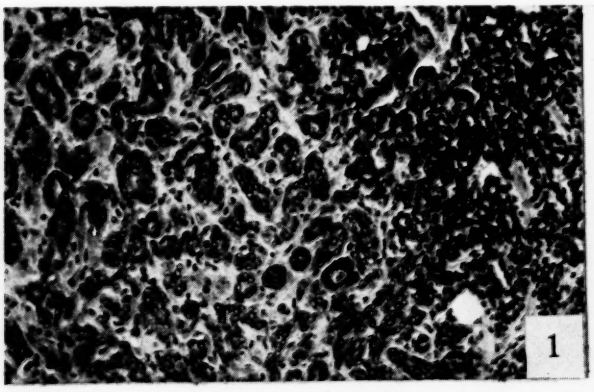
Type of lesion	No. of mice	Totals
I. Mammary gland carcinoma		45
a. Usual adenomatous carcinoma of mice	40	
b. Scirrhous type	5	
c. Squamous metaplasia (These mice included under a.)	11	
II. Mammary gland carcinoma, alone or associated with other tumors		45
a. Alone, with no other tumor	21	
b. With spindle cell sarcoma only	12	
c. With carcinoma of the skin only	8	
d. With lung tumor and rhabdomyosarcoma	1	
e. With spindle sarcoma and carcinoma of the skin	3	
III. Nonmalignant hyperplasia of mammary gland		9
a. With spindle cell sarcoma	7	
b. With carcinoma of the skin	0	
c. With spindle cell sarcoma and carcinoma of the skin	2	
IV. Squamous metaplasia in mammary gland carcinoma		11
a. With carcinoma of the skin	3	
b. With spindle cell sarcoma	0	
c. In mammary gland carcinoma alone, no other tumor in animal	8	

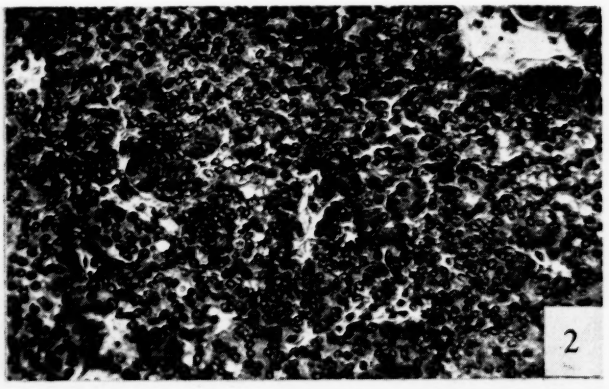
The average age of those mice which developed mammary carcinoma alone was 159.8 days following the injection of the methylcholanthrene (146.6 days for those which showed squamous metaplasia; 167.9 days for the adenomatous type). The average age of mice showing both mammary tumors and an associated other type of tumor was 146.1 days. Forty-two of these mammary tumors arose at sites adjacent to injection points (locally induced); 3 on the opposite side of the body (remote induction).

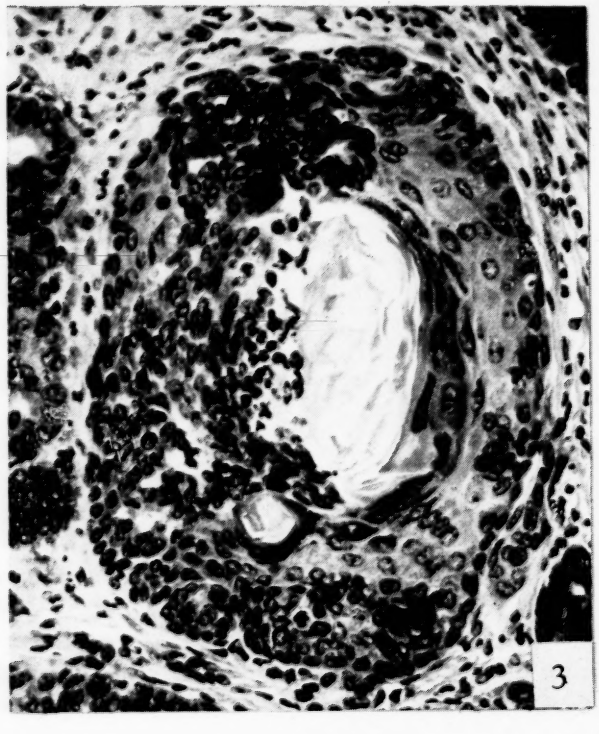
General Discussion

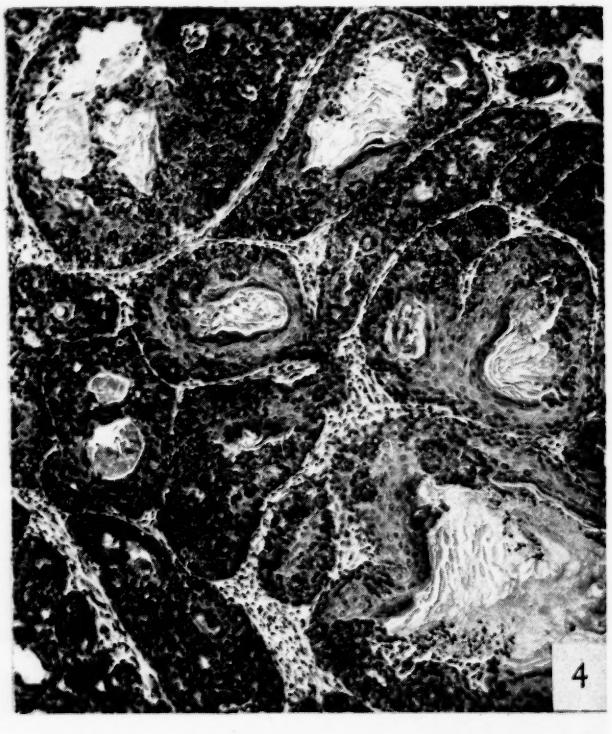
The occurrence of mammary gland changes as a result of the injection of estrogens in both male and female mice parallels genetic susceptibility, as stated in reviews by Gardner (9) and Loeb (12); that is,

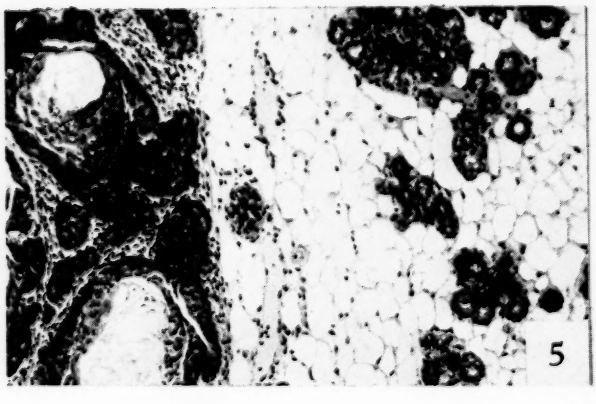
[1] The term collision tumors is frequently applied to such tumors involving more than one type of neoplasia. Since, however, collision implies physical contact especially of a violent nature, the term as applied to these tumors is, perhaps, an inappropriate one.

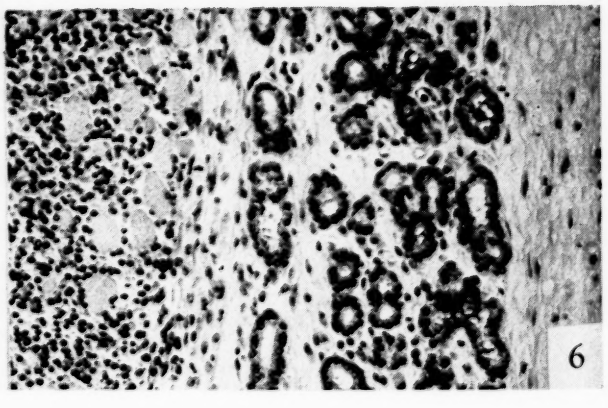












FIGS. 1 and 2.—The more typical mammary tumors which occurred in the mice of the NH strain. Mag. × 100 and × 150, respectively.

FIGS. 3 and 4.—A mammary tumor which has undergone considerable squamous metaplasia. Note polymorphonuclear leukocytes and cornification. There were no skin lesions in this animal. Mag. × 320 and × 100, respectively.

FIG. 5.—Subcutaneous area of mammary hyperplasia, adjacent to a metaplastic (squamous) mammary carcinoma. No skin lesions in animal. Mag. × 100.

FIG. 6.—Focal area of mammary hyperplasia adjacent to a spindle cell sarcoma. Mag. × 150.

the full development of neoplasias of mammary origin in a male mouse has not, with few exceptions, been brought about by estrogens except in mice of strains highly susceptible to carcinoma. There are no reports of the production of mammary tumors by estrogen treatment alone in mice belonging to a strain genetically resistant to cancer. Thus it is indicated that the response to the estrogen may serve as a measure of genetic susceptibility. Twombley (22), however, with a combination of foster nursing and estrogen treatment, has been able to obtain carcinoma of the mammary gland in male mice of the C57 black (cancer-resistant) strain. Other criteria, however, such as (a) tolerance to salicylaldehyde (17, 19), (b) changes in hemoglobin levels at different ages (15), and (c) abundance of porphyrins in the Harderian glands (20) also parallel and may be used as an index of genetic susceptibility to carcinoma.

That a possible estrogenic effect of the carcinogen used may be a factor in the origin of carcinoma of the mammary gland of this series must be taken into consideration. Cook and Dodds (7) reported that 100 mgm. of three phenanthrene derivatives (9,10-dihydroxy-9,10-di-n-butyl-9,10-dihydro-1,2,5,6-dibenzanthracene, 5,6-cyclopenteno-1,2-benzanthracene and 1,2-benzpyrene) dissolved in sesame oil brought about estrous in mice. They conclude that since massive doses were used the reaction must be considered qualitative and not quantitative as compared with estrone. Carminati (6) was unable to find estrogenic activity with 1,2-benzpyrene in rats. Perry (13), however, using 31 mgm. of 3,4-benzpyrene (1,2-benzpyrene) in 0.75 cc. of lard was able to report cornified vaginal smears within 48 hours of the subcutaneous injection of this carcinogen. In view of (a) the massive doses used and (b) the discrepancies of results obtained by several investigators in measuring estrogenic activity of carcinogens, it is perhaps unwarranted to maintain that the induction of mammary carcinomas in this present series was brought about by an estrogenic effect of the carcinogen upon mammary tissue (only 1 mgm. of methylcholanthrene was injected once and the tumors arose later after several weeks). Lewis and Turner (11) have, perhaps, indicated a possible explanation in that they find that 1.5 mgm. of 1,2,5,6-dibenzanthracene, even though non-estrogenic, will bring about mammary growth. They conclude that, "in this particular it compares with the mammogenic duct growth factor of the anterior pituitary."

The present experiment may indicate another mechanism in the origin of mammary tumors. So far these mammary tumors have occurred only in female mice of cancer-resistant strains, which have been used for breeders. Whether they would arise in virgins of the same strain is not known as yet. There is sufficient evidence available to demonstrate that mammary tumors never arise in male mice of the NH strain after the injection of methylcholanthrene. On the other hand, the induction of mammary tumors in breeder female mice (45 out of 384 or 11.7 per cent) is highly suggestive. That these tumors have been obtained in three strains (JK, NH, and F), all of which are highly *refractory to the spontaneous occurrence* of mammary tumors, indicates that another mechanism than the one accepted for the induction of mammary tumors by estrogens may be operating. It is not likely (although not impossible) that the presence of methylcholanthrene should take the place of genetic susceptibility. Neither does it seem likely that methylcholanthrene could destroy or inactivate genetic resistance to mammary tumors.

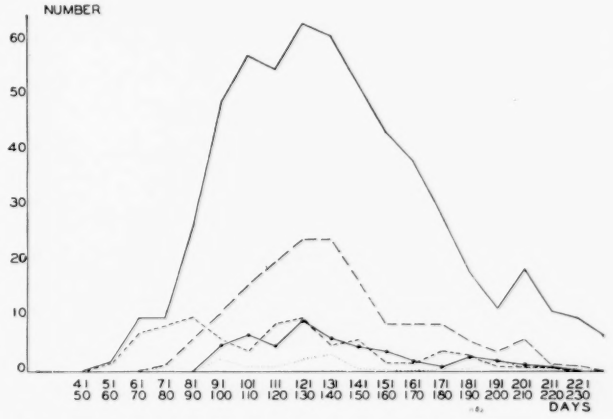


FIG. 7.—This chart shows the frequency distribution of specific types of tumors induced in 800 mice of the NH strain by 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil, as follows: (a) solid line—total number of tumors up to 240 days following the injection of the carcinogen; (b) long dash line—spindle cell sarcoma unaccompanied by any other tumor; (c) short dash line—carcinoma of skin unaccompanied by any other tumor; (d) solid line and solid dot curve—carcinoma of skin and spindle cell sarcoma; and (e) dotted line—carcinoma of mammary gland, occurring alone and associated with another type of tumor.

The present experiment also suggests that in the origin of the spontaneous tumors of mammary tissue there may be a carcinogenic agent which carries the process of oncogenesis further than does the intrinsically produced estrogen. In other words, the estrogen stimulates mammary tissue and maintains the pubertal and gestational growth phases but some other agent initiates the true neoplastic changes in the physiologically altered (estrogen-stimulated) mammary tissue. Such a concept would bring together the observations on (a) spontaneous mammary tumors arising in different genetic strains, (b) occurrence of tumors of similar origin in both males and females by estrogens (only in genetically susceptible strains), (c) the induc-

tion of similar tumors by methylcholanthrene in genetically resistant strains, and (d) the "physiological use" factor of Bagg (1).

Summary

In a series of 800 mice of the NH descent injected with 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil at 60 days of age, 45 showed definite evidence of carcinoma of the mammary glands. These carcinomas all occurred only in female mice. In addition to these neoplasms, hyperplasia of mammary tissue and squamous metaplasias of mammary tumors were also found in the treated animals. These types of mammary tissue response occurred separately and in combination with other types of tumor, such as (a) spindle cell sarcoma, (b) carcinoma of the skin, and (c) rhabdomyosarcoma. Mice of the NH descent are characterized by showing a high resistance to spontaneous tumors of mammary origin.

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An Effect of Heredity on the Susceptibility of Rats to Implants of an Induced Sarcoma

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The effects of heredity on transplanted tumors have been studied extensively, and several reviews of the subject have been published (5, 6, 14, 15, 22, 25, 26). The greater part of the work on hereditary aspects of the cancer problem has been done with inbred strains of mice. Studies using rats as experimental animals have been much less extensive, both with regard to the number of standardized strains and amount of work done.

Since the publication of the reviews cited above, the work with mice has strengthened the position of importance given to heredity in cancer research. Andervont (1-3) has used pure strains of mice for the induction and transplantation of tumors, and has concluded that the genetic constitution of the animals is of prime importance for the successful transplantation of induced as well as spontaneous tumors.

Lewis and Lichtenstein (13) also found that genetic constitution is important in transplantation of induced tumors. Two hundred dibenzanthracene-induced tumors, induced in pure inbred strains of mice, all proved to be transplantable into mice of the same strain. One hundred and fourteen of these tumors were transplanted into strains other than the one in which they originated, but only 9 grew in animals of foreign strains. It was further found that 14 of the tumors lost their strain specificity after repeated implantation into other strains. This last piece of evidence was in accord with the earlier work of the authors (11, 12) in which they found that it was possible to break down the resistance of strains of mice to induced tumors from other strains by repeated inoculation. It was found (13), however, that all of the spontaneous mammary tumors transplanted were strain-specific.

Lewis (10) later stated that immunity to a tumor could be induced by a 4-day growth of the tumor in a strain other than the one in which it originated, but that immunity failed to develop when the tumor was transplanted into mice of the same strain.

Barrett (4) has found that no significant resistance could be induced in an inbred mouse against a tumor derived from the same strain. Furthermore he concluded that resistance produced by subcutaneous injection of defibrinated whole blood was dependent upon the genetic interrelationship of the host, the tumor, and the donor of the blood.

The question of growth rhythms in tumors of mice has been studied recently by Selbie (21). He presented evidence indicating that the fate of a tumor graft is dependent to a great extent upon the hereditary susceptibility of the animal into which the tumor is transplanted.

* Departmental paper No. 355.

Reports on the use of rats published before 1929 have been covered by Woglom (25) in his review of transplantable tumors. Brief references to some of these reports will be given here. Levin and Sittenfield (9) demonstrated the possibility of heredity as the determining factor in resistance to tumor transplantation. They thought resistance to be due to a single Mendelian dominant. Roffo (17, 18) reported on a large number of rats and was impressed with the constant predominance of Mendelian laws as manifested in the susceptibility to inoculated tumors.

The only article which did not uphold the theory of heredity in tumor transplantations was that of Morpurgo and Donati (16). However, since the number of animals used was small and the degree of inbreeding was not definitely stated, the results are questionable.

The work with rats published since Woglom's review has continued to add evidence which indicates that hereditary factors influence the growth of transplanted tumors. Chambers and Scott (7) noticed that in their colony there had been a definite change in susceptibility to transplantation of the Jensen rat sarcoma. This change was brought about by a change in the technic employed. In their earlier experiments, animals which grew tumors progressively were operated upon and the tumors extirpated. These rats, along with those which failed to grow tumors, were used for breeding. In the later experiments only those animals were used for breeding which failed to grow tumors. It was then found that the number of resistant animals had increased from 6.1 to 54.6 per cent during 4 years. Although the Jensen sarcoma has been reported to be variable in its growth, such a reduction in susceptibility would be explained most logically on the basis of transmission of resistance from parent to offspring.

Russ and Scott (19) have presented evidence that the offspring of immunized rats are more resistant to the growth of the Jensen rat sarcoma than are the normal controls. Breeding rats were immunized to Jensen sarcoma by growth and regression of the implanted tumor. Transplants were made into the offspring born before and after the immunization, and it was found that those born before were more susceptible than those born after immunization. The evidence is presented as proof of the inheritance of acquired immunity, and will be of great importance if it can be confirmed.

Strong (23) in 1926 reported work in which he had used market mice to attempt to find some mice that were resistant to a "nonspecific" tumor. Out of 500 mice into which tumors were transplanted, he obtained 3 mice that were resistant. From these mice he was able to breed a strain that was completely resistant to the same tumor.

Selbie (20, 21) in 1936 reported that he had been able to change the susceptibility of a colony of rats toward the Jensen rat sarcoma from 46 per cent to over 93 per cent in a period of 4 years. This change was accomplished by using for breeding

stock only those animals that had had large progressively growing tumors removed.

The following report is a study similar to those of Strong and Selbie cited above. It deals with the response of groups of rats toward the implants of an induced sarcoma.

Procedure

During an experiment on the induction of tumors by 20-methylcholanthrene and 9,10-dimethyl-1,2-benzanthracene in various solvents (8), many of the tumors produced were transplanted in an attempt to find one which would grow progressively in a high percentage of the rats. Of the 46 sarcomas transplanted, about half were lost in the first passage because of failure to grow, but 7 were carried through 3 passages and might have been carried indefinitely had a sufficient number of recipients been used. Table I gives the results of transplanting the various tumors, and shows the irregularity of results to be

Table I: Results of Transplanting Induced Sarcomas into White Rats of Heterogeneous Ancestry

		1st passage		2nd passage		3rd passage		4th passage	
Carcinogenic agent	Tumor No.	No. rats transplanted	No. positive	No. rats transplanted	No. positive	No. rats transplanted	No. positive	No. rats transplanted	No. positive
Methylcholanthrene	1	4	0						
	2	5	1						
	3	2	0						
	4	6	1						
	5	5	0						
	6	3	0						
	7	10	0						
	8	3	0						
	9 *	4	4	39	14	36	19	38	27
	10	4	0						
	11	4	0						
	12	8	3	8	8	10	3	..	..
	13	4	0						
	14	23	0						
	15	10	1	18	5	22	11	..	..
	16	4	2						
	17	4	0						
	18	27	0						
	19	13	0						
	20	10	2						
	21	14	6	9	6	8	2	..	..
	22	18	5						
	23	21	4						
	24	15	0						
	25	10	0						
	26	17	0						
	27	14	10						
	28	6	6	8	8	7	4	..	..
	29	16	0						
	30	7	0						
	31	15	0						
	32	7	0						
	33	10	0						
	34	15	2						
Dimethyl-benzanthracene	35	6	0						
	36	15	11						
	37	10	0						
	38	11	1						
	39	4	0						
	40	17	2						
	41	26	15						
	42	19	8	11	6	9	9	10	9
	43	14	3						
	44	10	8						
	45	10	5						
	46	25	4	8	5	8	1	..	..

In the first passage there were 505 recipients and only 104 were positive; in the second, third, and fourth passages there were 249 recipients and 137 positive.

* This tumor was used in the subsequent division of the colony into two strains, one resistant and one susceptible.

expected when transplantation is made into animals of heterogeneous origin.

The final selection of the tumor to be used for continued transplantation was based on its histological stability as judged from microscopic examination of successive passages, its regularity of growth without excessive necrosis, and the ease of propagation. This tumor, our No. R-9, was a moderately well differentiated fibrosarcoma which gave about 55 per cent takes in the first 4 passages in rats weighing 60 to 120 gm. The first 4 passages were made into 117 animals before selective breeding was started.

Early in the course of the transplantation experiments it was noted that susceptibility and resistance were peculiar to certain litters, and not a haphazard distribution. It was then decided to attempt to divide the colony into susceptible and resistant lines. The animals were selected from a group of rats of heterogeneous ancestry which originated from 4 males and 15 females and, at the time of making the selection, the entire colony consisted of about 300 individuals.

The breeding was started by brother and sister, father and daughter, or mother and son matings among the susceptible or resistant animals. The use of the litter as the unit for judging the value of resistance or susceptibility had been suggested in 1913 by Sweet, Carson-White, and Saxon (24).

All of the offspring of both susceptible and resistant animals were tested by transplantation with the R-9 sarcoma. The offspring of the susceptible animals were observed for a period of 4 weeks, and if at the end of this time there was no evidence of regression, the tumors were extirpated and the animals saved. The offspring of the resistant animals were observed for a period of 4 to 5 weeks, and if at the end of this time there were no tumors present, the animals were considered as having fulfilled the requirements of resistivity. The more vigorous of the animals tested were then selected for use as breeders.

As the study progressed, we became interested in the response of successive generations toward the tumor and in the degree of success that could be obtained in the development of two strains, one resistant and the other susceptible. Each strain was started in the following manner: Strain S (susceptible) was established by arbitrarily selecting 8 pairs from litters which gave the highest incidence of takes and progressive growth of implants. Strain R (resistant) was established in a similar manner from litters showing the least incidence. Records of the succeeding generations from each of the original pairs were kept, and the progeny resulting from close inbreeding designated as a substrain. Some of the substrains died out rather promptly as a result of nonbreeding, and others were discarded because they lacked vigor or were not sufficiently prolific. At the present time, 3 substrains of strain S and 2 substrains of strain R remain.

Results and Discussion

Tables II and III give the number of rats in each substrain into which sarcoma R-9 was transplanted. Nearly all of the animals received four implants, but a few of the first animals transplanted received only two. The animals were examined weekly for 4 to 5 weeks and the data recorded are based on their condition at the end of this period.

Substrain 22 shows that resistance can be almost complete, since the tumor was able to grow in only one animal in 45. In this one animal only one implant of the four was present at the end of 5 weeks. Substrains 8 and 23 show much less perfect resistance, and nearly one-third had tumors present at the end of 5 weeks.

Few resistant animals appeared in strain S, and these were limited to substrains 2 and 7. The incidence of susceptible animals was 100 per cent in the other six substrains. The number of implants which grew progressively in strain S animals varied from 83 per cent in substrain 7 to 98 per cent in substrain 3. A similar comparison among the substrains of the resistant strain R shows that from 0 to 42 per cent of the implants grew. There was therefore no tendency toward overlapping of the results obtained within the two strains.

The data are of interest chiefly in terms of the totals, since the number of animals in several substrains is not large enough for conclusions. The totals show that the simple selection of breeding pairs from the original colony on the basis of their susceptibility or resistance revealed strains which differed markedly in response to the tumor. This seems to indicate that the factors for susceptibility and resistance were fairly well aggregated in the individuals chosen for breeding, because the reactions of the first generation of offspring were about the same as those of subsequent generations. The phenomenon, as we have seen it, is not specifically associated with progressive selective breeding but, presumably, a more nearly perfect degree of resistance will be obtained by continued selective breeding.

There is, of course, a certain amount of error inherent in an experiment of this type. It is possible that somewhat different results might be obtained if it were possible to allow the transplants to grow in the susceptible animals indefinitely. However, it was necessary to set the time of the extirpation of the tumors arbitrarily in order to save the susceptible animals for breeding. Thus it was not possible to allow the tumors to grow to a terminal condition.

Such continuation of the time of observation might have disclosed a few regressions, but from earlier observations on the behavior of the tumor we believe the number would have been too few to have any significance.

Summary and Conclusions

1. The results obtained by transplantation of any particular induced tumor into a group of genetically heterogeneous rats are very irregular and unpredictable.

2. It is possible to separate a group of rats of heterogeneous origin into two strains, one susceptible and the other resistant to a certain tumor, by careful selection and testing of the breeding stock.

3. The response of rats to a transplantable induced sarcoma is determined largely by heredity.

4. In our colony, types both resistant and susceptible to a particular sarcoma were present and tended to breed true after simple selection on the basis of litter response.

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Table II: Data on the Transplantation of Sarcoma No. R-9 into Rats of the Resistant Strain

Substrain No.	No. of recipients: No. males	No. of recipients: No. females	No. of positives: No. males	No. of positives: No. females	No. of implants	No. of tumors	Percentage of implants that grew
8	16	25	3	9	150	34	23
9	4	2	0	0	24	0	0
10	3	3	0	0	24	0	0
11	0	4	0	1	16	3	19
13	4	1	1	1	20	5	20
14	2	4	2	2	24	10	42
17	7	8	3	5	60	16	27
19	8	2	2	1	40	11	28
22	19	26	0	1	180	1	0.6
23	53	60	16	19	452	93	21
24	6	6	4	1	48	14	29
Totals	122	141	31	40			
Grand totals	263		71		1,038	187	

Of the 263 animals transplanted, only 71 or 27.0 per cent grew tumors. Of the 1,038 implants made, 187 or 18.0 per cent grew.

Table III: Data on the Transplantation of Sarcoma No. R-9 into Rats of the Susceptible Strain

Substrain No.	No. of recipients: No. males	No. of recipients: No. females	No. of positives: No. males	No. of positives: No. females	No. of implants	No. of tumors	Percentage of implants that grew
1	6	11	6	11	58	56	97
2	7	5	6	5	38	32	84
3	23	19	23	19	268	263	98
4	1	6	1	6	28	27	96
5	5	3	5	3	32	31	97
6	38	32	38	32	280	262	94
7	28	19	25	15	188	156	83
25	10	7	10	7	68	65	96
26	5	3	5	3	32	31	97
Totals	123	95	119	91			
Grand totals	218		210		892	823	

Of the 218 animals into which transplants were made, 210, or 96.8 per cent, grew tumors progressively. Of the 892 transplants made, 823, or 92.3 per cent, grew progressively.

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The Relationship of Twins, Teratomas, and Ovarian Dermoids

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Introduction

The bizarre structure of teratomatous tumors has long excited interest. Unlike other neoplasms, the teratomas are composed, characteristically, of more than a single type of cell and are related, histogenetically, to more than one of the three embryonic germ layers. The constituent elements of these tumors may be quite foreign to their site of origin in the body and, in their arrangement, tend to mimic organs or even organ systems. The abortive or imperfect character of this resemblance finds reflection in the name, first applied by Virchow (15), of "teratoma," a malformed or "monstrous" tumor.

It is natural that the morphologic peculiarities of the teratomas should have led to the formation of theories of pathogenesis differing from those associated with other tumors. With its pleomorphic, organoid character, a teratoma represents in itself, as expressed by MacCallum (9), "a frustrated attempt at the formation of a human body in which the whole plan has failed through the lack of the necessary parts, and the distortion and disarrangement of those which were available."

Two alternative mechanisms might reasonably explain the occurrence of such an ontogenic fiasco. First, there is abortive parthenogenesis. Second, there is inclusion of isolated blastomeres.

A teratoma might be the end result of spontaneous development of unfertilized germ cells, ova or sperm. Several facts are in favor of such a view. The gonads are among the commoner sites occupied by tumors of this group. Dermoids comprise approximately 18 per cent of proliferative ovarian neoplasms (14), while tumors of the testis are quite commonly teratomatous. Michalowsky (11) and Bagg (1) have produced teratomas of the testes in the rooster, by the injection of zinc chloride solutions. Bosaeus (2) demonstrated the production of teratomas in frogs by the reimplantation into the mother's body of eggs that had been removed and mechanically stimulated to develop parthenogenetically. Earlier, Loeb (7) described the occurrence of teratoid structures in the ovaries of virgin guinea pigs, and pointed out their importance as putative links between unfertilized ova and true teratomas, evolved through parthenogenesis.

A teratoma might also be formed by the isolation and inclusion of a blastomere in the body of the embryo that develops into the host of the teratoma. Segregation of such a relatively undifferentiated cell, or group of cells, with temporary inhibition of its development, might result in the formation of a "cell rest," in the sense of Cohnheim (3) and Ribbert (12), that would have sufficient multipotency to produce a teratoma, once it became reactivated. This theory, too, gains support from diverse facts. As emphasized by Marchand (10), Schwalbe (13), Greil (5), and others, it is possible to construct complete morphologic series of cases ranging from equal conjoined twins through irregular parasitic conjoined twins, and through the more complex embryomas to the characteristic teratomas. So many intergrades exist, anatomically, between twins and teratomas, that one might naturally expect to find common, or at least similar, modes of production of twins and teratomas. While knowledge of the actual mechanism of human twinning is still inferential, it seems most likely that the process involves a splitting of the zygote with developmental separation of component portions. Whether "identical" or "fraternal" twins are produced by similar or by dissimilar mechanisms is an important, but unsettled question. Curtius and von Verschuer (4) believe the mechanisms are similar; most other authors believe them dissimilar. At least one type of twin, however, is formed by a process so closely analogous to that postulated by the inclusion theory of teratoma formation, that such variables as time of fission or relative proportions of the divided segments offer themselves as logical modifiers determining whether twin or teratoma is the end result.

The teratomas encountered in extragonadal locations are most frequently situated in or near the midline of the host. The sacrococcygeal region is a classic site. It seems hardly coincidental that the midline is a common axis of symmetry in cosmobia (equal and unequal) or that the sacrococcygeal region is an equally classic site of fusion of conjoined twins.

The occurrence of teratomas in infants at an age when developmental inclusions would seem more plausible than abortive parthenogenesis, is a final point in favor of the inclusion theory.

There remains a third possibility. There may be, as suggested by MacCallum (9), more than one type of teratoma, referable to more than one mechanism of production. Perhaps ovarian dermoids are produced parthenogenically while sacrococcygeal teratomas are the result of blastomeric segregation and inclusion.

If a relationship between twins and teratomas is possible, it would seem reasonable to enquire into the twin-like character of teratomas. The influence of heredity in twinning seems established by several statistical studies included in the literature reviewed by Greulich (6), although the mechanisms involved are uncertain. Is heredity, then, a factor in the production of teratomas? Reports of familial teratomas are rare (8). If, however, twins and teratomas depend on similar developmental mechanisms, may not a common factor of heredity be involved? If so, twinning would be associated with teratoma formation in families; twins would occur in the families of patients with teratomas more frequently than in a nontwinning or random population.

Materials and Methods

With the above considerations in mind, patients with two types of teratomatous tumors and appropriate control patients have been investigated with respect to the occurrence of twins in their families. The tumor types selected were ovarian dermoids, and teratomas occurring in childhood.

The term "ovarian dermoid," as used in this study, refers to a cystic tumor of the ovary which, on pathologic examination, was found to contain hair and sebaceous material, frequently associated with additional structures such as teeth, cartilage, or glandular tissue.

The term "teratoma," as used in this study, refers to a cystic or solid tumor which, on pathologic examination, was found to contain more than one tissue element—nerve and muscle, hair and cartilage, etc. Eighteen of the teratomas included in the present study were situated along the spinal axis, while the following localities were involved in a single case each: mediastinum, retroperitoneal space, abdominal wall, pleural cavity, nose, and eyelid.

One of us (H.W.E.) has collected a series of 50 patients with ovarian dermoids, a series of 50 patients hospitalized for twin pregnancy, and a series of 40 patients hospitalized for singleton pregnancy. Each series was consecutive. In each case investigated, a pedigree was obtained from the propositus at a single interview in the hospital. The patients comprising the first series were located through the pathologic laboratories of the Barnes, Maternity, and Jewish hospitals of St. Louis, of the Lying-In, City, Massachusetts General, and Peter Bent Brigham hospitals of Boston, and of the Free Hospital for Women in Brookline, Massachusetts. The patients of the second series were obtained similarly from the Maternity and the DePaul hospitals of St. Louis, and from the Lying-In Hospital of Boston. The entire third series was obtained from the last named institution. The authors offer their sincere thanks to the respective pathologists of these hospitals for their kind permission to carry on this study, and to their residents and secretaries for their invaluable assistance in securing interviews with the patients. Data were collected during the years 1935 through 1941, inclusive.

One of us (J.W.H.) has collected a series of 24 child patients with teratoma, a control series of 26 children having similar socio-economic status, and a control series of 24 students in the Harvard School of Public Health. In the first two series, pedigrees were obtained by interviews, sometimes repeated, with the parents and grandparents of the propositi in their homes. In the third series, pedigrees were obtained by questionnaires, filled in by the propositi. The cases of the first series form part of the collection of tumors of early life now under study in the Department of Pathology of the Children's Hospital of Boston. Data in this part of the study were collected in the years 1939 through 1941, inclusive.

The incidence of twins in these families was determined by two methods. The simplest method was based on the presence or absence of twins in each family, the family including, in this instance, blood relatives. The results are expressed in terms of the percentage of families with twins. The more detailed method of analysis consisted of determining the total number of twin births, the total number of single births, and of expressing the results in terms of the rate of twin births per thousand total births.

Ovarian Dermoids

The significant data obtained from the first three series are presented in Tables I and II. The first tabulation indicates the presence or absence of twinning in the entire family of the propositus, while the second records the rate of twinning in the sibship of the propositus. It is evident that occurrence of twins in the family is more common with patients with ovarian dermoids and with patients with twin pregnancy than with the random sample of population represented by the patients with singleton pregnancy. Both dermoid and twin series diverge from the singleton series by differences of similar magnitude. While these differences fail to exceed twice their

standard errors, owing to the smallness of the populations employed, they are probably real, because they correspond to differences obtaining in another type of analysis, twin birthrate method. The differences between the dermoid and twin series on the one hand, and the singleton series on the other, in regard to rate of twin births (Table II), are striking. That the respective differences between the series, analyzed by these two separate methods, should so agree is a fact that adds to the significance of the figure.

Teratomas in Children

The significant data obtained from the second three series are represented in Tables III and IV.

Table I: Incidence of Families* with Twins in Ovarian Dermoid Series and in Control Series

Series	No. of families studied	No. of families including twins
Dermoid	50	28 (56%)
Twin pregnancy	50	†30 (60%)
Singleton pregnancy	40	16 (40%)

* "Family" as used in this table and accompanying discussion refers to the entire collection of blood relations included in the pedigree.

† Obviously every family in the twin series included one set of twins, the twin birth for which attention was drawn to the propositus. Only families with additional sets of twins, other than the initial set, have been credited in this table.

Table II: Twin Birthrates in Sibship of Propositus in Ovarian Dermoid Series and in Control Series

Series	Rate per 1,000 births
Dermoid	22.0
Twin pregnancy	18.0
Singleton pregnancy	5.3

In these tabulations, the two control series (families of children having socio-economic status similar to that of the children with teratomas, and families of students in the Harvard School of Public Health) were similar and therefore have been pooled. It is evident that the families of teratomatous children include twins more frequently than do the families of the individuals of the random control series. Further, the rate of twin births is higher in the former series than in the latter. Again, the similar divergences between the two series, in regard to the separate considerations of presence of twinning in the families and of twinning rate, mutually strengthen their significance. With the more extended pedigree information obtained in this part of our study, twin birthrates for the sibships of the parents of the propositi are available. As recorded in Table IV, there are equally impressive differences in rates between the tumor series and the control series.

Discussion

Twin birthrates in the ovarian dermoid series, in the childhood teratoma series, and in the several control series may be compared with the twin birthrate reported by the United States Census. This later rate, 11.5 per thousand (a value exceptionally stable, since derived from a population representing a statistical "universe") is very similar to the rate for the control series of mothers hospitalized for singleton pregnancy, and the rate for control nontumorous children and students of the Harvard School of Public Health, indicating adequate control. The twin birthrates obtained for the twin pregnancy series, for the ovarian dermoid series, and for the childhood teratoma series are all similar and are all much in excess of the controls.

Table III: Incidence of Families with Twins in Teratoma Series and in Pooled Control Series

Series	No. of families studied	No. of families including twins
Teratoma	23	19 (83%)
Control *	50	17 (34%)

* The mode of selection of the control group is detailed in the text.

Table IV: Twin Birthrates in the Families of the Teratoma Series and in Pooled Control Series

Series	Rate per 1,000 births	
	Sibship of propositus	Sibships of parents
Teratoma	21	18
Control *	12	11

* See note, Table III.

Our cumulative data indicate a high incidence and rate of twinning in the families of patients with ovarian dermoids and in the families of patients with childhood teratomas, similar to the high incidence and rate of twinning in the families of mothers giving birth to twins.

This evidence is consonant with the idea that factors of heredity similar to those conditioning twinning may also condition the occurrence of ovarian dermoids and of teratomas of children. It favors the idea that ovarian dermoids and the teratomas of children are at least partly alike in mode of formation. Finally, a certain amount of support is given to the inclusion theory of the pathogenesis of the teratomatous tumors.

Conclusion

The incidence of twinning, whether measured by the percentage of families with twins or by the ratio of twin births to total births, is similar and con-

sistently high in families of patients with ovarian dermoids, of patients with childhood teratomas, and of patients with twin pregnancy—higher in each instance than in random control groups. This evidence supports a theory of similarity in pathogenesis of teratomatous tumors and of twins, probably involving common factors of heredity.

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The Incidence of Carcinoma of the Lung

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This study was undertaken to collect further information concerning the frequency with which carcinoma of the lung is encountered at necropsy. In a previous communication the material of the Charity Hospital of Louisiana at New Orleans was surveyed (1). In this paper a like study covering the same period is presented on the necropsy material of the Department of Pathology, University of Chicago.[1]

During the decade ending December 31, 1940, a total of 3,014 necropsies was performed, 2,781 of which were on persons over one year old. Among these subjects there were 74 with carcinoma of the lung, 115 with carcinoma of the stomach, 30 with carcinoma of the biliary system, and 33 with carcinoma of the pancreas. Carcinoma of the lung was therefore more than one-half as frequent as carcinoma of the stomach and more frequent than carcinoma of the biliary system and carcinoma of the pancreas together. A year-by-year analysis is shown in Table I.

TABLE I: REGIONAL DISTRIBUTION OF CARCINOMA, DEPARTMENT OF PATHOLOGY, UNIVERSITY OF CHICAGO—1931-1940

	Necropsies on persons over one year old	Lung	Stomach	Biliary system	Pancreas
1931	264	8	7	2	3
1932	242	5	13	3	2
1933	233	4	7	0	4
1934	263	6	8	2	1
1935	293	7	12	6	3
1936	314	7	9	3	4
1937	286	7	10	5	3
1938	312	15	20	3	5
1939	277	6	12	3	4
1940	297	9	17	3	4
Total	2,781	74	115	30	33

Race, sex, and age incidence.—Of the 2,781 necropsies, 1,792 were performed on male subjects (1,729 white, 63 negro) and 989 on female subjects (975 white, 14 negro), a proportion of males to females of almost 2:1. Carcinoma of the lung, however, occurred in 59 men (56 white, 3 negro) and 15 women (all white), a ratio of males to females of almost 4:1.

The youngest patient was 32 and the oldest 83. Sixteen died in the fifth, 34 in the sixth, and 19 in the seventh decade of life or older. More than 70 per cent of the patients were thus in the sixth decade of life or older (Table II).

Cellular structure.—Among the 74 carcinomas of the lung, 32 (43.2 per cent) were squamous cell, 23 (31.1 per cent) were reserve cell, and 19 (25.7 per cent) were columnar cell carcinomas (Table III).

TABLE II: AGE INCIDENCE OF CARCINOMA OF THE LUNG

Age in years	Male, white	Male, negro	Female, white	Female, negro	Total
31-40	2	0	3	0	5
41-50	12	2	2	0	16
51-60	29	1	4	0	34
61-70	9	0	4	0	13
71-80	4	0	1	0	5
81-	0	0	1	0	1
Total	56	3	15	0	74

TABLE III: TYPE AND INCIDENCE OF CARCINOMA OF THE LUNG ACCORDING TO RACE AND SEX

	Male, white	Female, white	Male, negro	Female, negro	Total
Squamous cell	26	5	1	0	32
Columnar cell	11	7	1	0	19
Reserve cell	19	3	1	0	23
Total	56	15	3	0	74
Necropsies	1,729	975	63	14	2,781

SUMMARY AND CONCLUSION

A survey of the records and material of the Department of Pathology, University of Chicago, disclosed that during the past decade 74 carcinomas of the lung were discovered in 2,781 necropsies on persons over 1 year old.

As in the necropsy material of the Charity Hospital of Louisiana at New Orleans for the same period, carcinoma of the lung was more than one-half as frequent as carcinoma of the stomach and more frequent than carcinoma of the biliary system and carcinoma of the pancreas together.

The data of this study support the assertion that carcinoma of the lung is becoming the second, if not the first, most common malignant neoplasm in the male.

[1] The writer is indebted to Dr. Paul R. Cannon for his permission to make this study and to Dr. Paul E. Steiner for his cooperation.

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Prevention of Cancer of the Vulva*

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Scientific investigation concerning the cause and prevention of cancer of the vulva has been retarded by the relative rarity of the disease in the human race, the absence of suitable animals for control experimentation, and the absence of records of leukoplakic changes and cancer of the sexual skin in monkeys. On the other hand, investigation of cancer of the vulva is facilitated by the superficial location of the growth. Like other cancers of the skin, its early development can be watched and studied in relation to preceding changes in the areas upon which cancer develops. If the general statement is true that cancer does not ordinarily develop from normal healthy tissues, extensive observations, generally accepted, indicate that this doctrine would seem to be particularly applicable in cancer of the vulva.

Clinical Observations

During the past 35 years I have observed 161 cases of cancer of the vulva. Of these, 101 patients had borne children, 42 were nulliparae, 11 were virgins, and in 7 sufficient data were lacking (1). Approximately an equal number of cases of leukoplakic vulvitis, a condition which has been generally considered a forerunner of this disease, were also observed during this period.

In a report (1) of 155 cancers of the vulva made last year the following possible etiologic factors were listed:

Leukoplakic vulvitis	72 cases
Syphilitic or postsyphilitic ulcers	9 cases
Senile warts	8 cases
Bartholin gland abscess	4 cases
Urethral caruncle	3 cases
Trauma	3 cases
No definite lesions	58 cases

It will be noted that in over one-third of the patients the history and findings gave no possible clew as to any etiologic factor. In the 3 cases of trauma we must acknowledge the uncertainty of the etiologic relationship, since a small lesion may have been present and the trauma merely served to accelerate the growth.

* Read at the 34th annual meeting, American Association for Cancer Research, Inc., Chicago, Illinois, April 15, 1941.

Urethral caruncle.—In urethral caruncle caused by chronic infection of the urinary meatus the relationship is more definite. In one patient the urethral caruncle was noted 4 years previous to the onset of the cancer of the urinary meatus. So far as I know no data are available on the frequency with which cancer develops from such a caruncle. Taking into consideration the relative incidence of these two conditions in practice, such a malignant change would not occur in over 5 per cent of the cases. In all such deductions we must guard ourselves against the fallacy of *"post hoc, ergo propter hoc."* It can, however, do no harm to excise or treat by radiation such chronically infected urethras. It is reasonable to suppose that to some slight degree the incidence of cancer may thereby be reduced.

Senile warts.—Senile warts are infrequently found. They must be distinguished from the relatively common acuminate warts associated with gonorrheal infection in younger individuals. The gonorrheal wart is typically cauliflower in structure with multiple small nodular branches. The so-called senile wart on the other hand is simpler in structure, with larger nodules, and covers a much smaller portion of the vulvar skin. It is a cluster of individual units rather than cauliflower in its shape. Senile warts are almost always found in older women of uncleanly habits. Whereas the ordinary acuminate warts usually appear and disappear within a relatively short period of time, usually a few months, in the patients with senile warts these excrescenses have existed for many years. Since so many of these women are not inconvenienced by these warts, they do not seek medical advice. Therefore, we have no accurate data as to the frequency of this lesion. Without having actually counted the number of cases of senile warts seen in my clinical experiences, I believe approximately one-half of these patients did not consult me until there had already developed at the site of these warty growths, a well-defined carcinoma. In the way of prophylaxis the same rules should apply to these vulvar warts as to warts in other portions of the integument. If the wart is subject to irritation or is enlarging it should be removed. On the vulvar skin these warts are apt to be abraded by the clothing and discharges in that area. In the 6

cases reported in my series, the patients stated positively that the warts had existed for months and years before the development of the ulcerating tumor. One might perhaps assume that both the warts and the cancer developed from a third unknown underlying cause. Nevertheless the appearance of such senile warts should have been a warning of possible trouble and hence should have justified their simple removal. The recognition of this etiologic relationship would doubtless serve in some cases to prevent and in others to make the early recognition of the disease more likely.

Abscess of Bartholin's gland.—Cancer of Bartholin's gland is extremely rare. There are only 9 cases in my series. The fact that in 4 of these 9 patients there had existed an infection of this gland so pronounced as to necessitate operative incision seems significant from an etiologic standpoint. In some of these patients there was a history of a residual infection in this organ. To suggest the routine removal of such infected glands in women past the menopause would seem a bit too radical, but certainly in routine follow-up examinations of women past the menopause this possible etiologic relationship should be kept in mind and in the presence of any pain or enlargement, the gland should be excised.

Syphilis.—The epithelial covering of the vulva can be divided into the *vestibular* area between the hymen and labia minora and the *epidermal* area comprising the remainder of the vulva. The latter has all the histologic characteristics of the skin, whereas the former resembles the squamous epithelium of the orifices. Cancer may arise from either site. Each has its distinctive etiology. In 9 out of 11 *vestibular* cancers, exclusive of those developing at the urinary meatus, there was a history of preceding syphilis. The diagnosis in all cases was established by typical skin lesions, positive Wassermann reactions, or a combination of the two. In 5 patients, the Wassermann reaction still remained positive. All of these women showed extensive destructive granulomas or hypertrophic masses in the region of the vulva or the rectum in addition to the cancer. It was difficult to decide how many of these should be classified as gummas and how many were lymphogranuloma venereum. The frequency of associated rectovaginal fistula and rectal strictures would point to the latter diagnosis. The Frei test was positive in 3 instances but 4 cases were observed previous to our present knowledge of such a test. Of the 9 patients with such syphilitic or postsyphilitic ulcerative granulomas associated with cancer, 8 were Negroes. From a standpoint of racial predisposition it is of interest that epidermal cancer of the vulva was very rare among the Negroes, only 2 such cases occurring in our series out of 161. One negress had a Bartholin gland cancer. It would seem, therefore, that syphilis and its sequellae, especially in the Negro race, play an important part in the etiology of vulvar cancer starting from the vestibular area. From a standpoint of cancer prevention, therefore, the treatment of these granulomatous ulcers, so common in the Negro race, is of great importance. Unfortunately, while we possess in the Frei test a reasonably accurate method of diagnosis, the cure of the granulomas with tartar emetic and other agencies is still far from effective.

Leukoplakia.—The five etiologic factors thus far mentioned are relatively infrequent and statements concerning them must be made with reservations. When it comes to leukoplakic vulvitis, its etiologic relationship to cancer of that region seems established beyond any reasonable doubt. And the corollary of this statement seems equally certain; namely, that by excising the leukoplakic area, we can often prevent the development of cancer at this point. This clinical observation was recognized in the nineteenth century. In 1898, J. Veit (2), writing in the *Handbuch der Gynäkologie,* records that from this atrophic vulvitis which he termed "Kraurosis," cancer eventually would develop in many instances. Hence, he says, the excision of the vulva was indicated not merely to relieve the pruritis but to reduce the chances for development of cancer.

In the series of 161 cases of cancer of the vulva thus far observed, 74, or approximately 45 per cent, showed evidence of a preceding leukoplakic vulvitis. Since during the same period approximately twice that number of cases of leukoplakic vulvitis were observed without evidence of carcinoma, we may infer that of the total number of cases of leukoplakic vulvitis, approximately one-third proceded to the development of cancer. These figures correspond to those given by others regarding the relationship between leukoplakic vulvitis and vulvar cancer. If we separate vulvar carcinoma according to its site, we find that in our series there were 107 persons in whom the cancer originated in the epidermis itself. Since in other forms of cancer (Bartholin gland, vestibulum, urethra) leukoplakic vulvitis did not enter as a possible etiologic factor, the incidence of 74 cases of leukoplakic vulvitis in 107 epidermal cancers has great significance.

Histologic studies of the tissues removed by vulvectomy in leukoplakic vulvitis and in cancer of the vulva associated with leukoplakic vulvitis tend to corroborate this etiologic relationship. As far as morphologic studies go, we can follow step by step in a series of cases (though of course not in the same case) the gradations between the break in the basement membrane in leukoplakic vulvitis to the well-developed carcinoma. In several cases the gross and

histologic picture has been such that I have been at a loss whether to call it cancer or not. Since in either event a vulvectomy was advisable, further observation was not possible. Leukoplakic vulvitis has many of the characters which we attribute to a precancerous condition, such as epithelial prolongations, breaks in the basement membrane, irregularity of cell shape, round cell infiltration, etc.

The comparison of leukoplakia of the mouth with leukoplakic vulvitis shows some similarities such as the piling up of the squamous epithelium with a tendency to the subsequent development of a cancer. There are, however, fundamental differences. In oral leukoplakia the process is essentially hypertrophic whereas in vulvar leukoplakia a markedly atrophic process with destruction of elastic tissue fibers more commonly precedes the formation of the keratotic areas from which in some instances cancer develops.

Out of the approximately 75 cases of leukoplakic vulvitis upon which I have actually done a complete removal of the lesion by partial or complete vulvectomy, one third, 25, would according to our figures have been expected to develop a carcinoma. By this operative excision of the diseased vulvar skin, it seems likely, therefore, that 25 cancers were prevented from development. However, even when a fairly complete vulvctomy has been done for the relief of the leukoplakic vulvitis and the prevention of a carcinoma, it is not certain that in every case this goal has been achieved, as seen in the following two cases:

Case 1.—Mrs. M. S. was operated upon by me at the age of 60 years for a leukoplakic vulvitis of long standing. A vulvectomy was done, but the pruritis returned one year later at a point just beyond the area of skin removed. Unfortunately the patient did not return for re-examination as she had been instructed. She was not seen from September 30, 1930, until November 25, 1936. By this time she had developed an epithelioma 4 cm. in diameter in the right labial region with evidence of leukoplakic change about it and the perineal region. The radical vulvectomy and double-sided Basset lymph gland removal was followed by a severe local infection aggravated by an acute cholecystitis, and death occurred 14 days after operation.

Case 2.—Mrs. K. M. (private patient of Dr. McNalley) consulted him in June, 1935, for pruritis vulvae. On October 29, 1935, a vulvectomy was done for a typical leukoplakic vulvitis. Microscopic examination at this time showed no evidence of cancer. In March, 1936, she was entirely well. She did not return for further observation until July 25, 1938, when examination showed a tumor 6 cm. in diameter in the right labial region rising from a remaining island of leukoplakia. The inguinal glands on the right side were enlarged and hard. A right-sided Basset operation was done and radium applied locally. The inguinal glands showed cancer. There was rapid extension of the disease and the patient died 3 months later.

From these two cases we must conclude that even after a fairly radical vulvectomy we should insist upon watching such patients at 6-month intervals for many years, and if it is found that an area of leukoplakia associated with pruritis has developed at some unremoved portion of the vulvar or peri-anal skin, this area should be freely excised to prevent the development of a cancer. In both cases above cited an interval of several years had elapsed with some return of symptoms and when the patient finally came for examination cancer was already far advanced. In at least 15 per cent of the vulvectomies for leukoplakic vulvitis we find a recurrence of the leukoplakia, but only very seldom does a cancer develop.

The etiologic relationship between leukoplakic vulvitis and cancer of the vulva is further illustrated by the cases in which the cancer is widely excised but some of the leukoplakic skin around the perineum and anus is allowed to remain. In 5 patients where such an island of leukoplakia was left or developed in later years, a new carcinoma began to grow at this point. In each instance the long interval after the removal of the original tumor and the gross and microscopic appearance of the new lesion left no doubt that we were not dealing with a metastatic recurrence but with a fresh neoplasm starting on a remaining leukoplakic area. In none of these 5 patients were there distant glandular metastases or evidence of other local implants. The relative frequency with which such new tumors developed points to a somatic factor in addition to the local irritant responsible for the tumor formation. Such cases emphasize the necessity of persistent re-examination in every patient who has had a leukoplakic vulvitis with or without the development of carcinoma.

In this connection it is important to point out the frequency with which we see multiple points of cancer development in vulvar leukoplakia. In some instances the lesions are so placed as to suggest a contact implantation but as a rule they are more widely separated. More than once a lesion about the clitoris has developed simultaneously with one over the perineo-anal skin.

Estrogens.—Mention should be made of the possible influence of the estrogens on the development of leukoplakic vulvitis and cancer of the vulva. The average age of patients with leukoplakic vulvitis in my series was 49 years and those with cancer was 59 years. Leukoplakic vulvitis is hardly ever seen before the age of menopause except in the presence of some ovarian disturbance or where both ovaries have been surgically removed. Cancer of the vulva in persons under 40 years of age is practically never on a leukoplakic basis. If an estrogen deficiency is responsible for the atrophic changes leading to the development of this so-called precancerous lesion, it is difficult to explain the cancer itself as due to a carcinogen of estrogenic origin.

Summary and Conclusions

Clinical observations on 161 cases of cancer of the vulva, seen in the author's practice during the past 35 years, are analyzed and discussed from the points of view of etiology and prevention. The chief conditions which appear to have etiological importance in cancer of the vulva are urethral caruncle, senile warts, abscess of Bartholin's gland, syphilis, and leukoplakia. This etiologic study points to certain pre-existing lesions tending to the development of malignancy, which if promptly removed may lead to an appreciable lowering in the incidence of cancer.

REFERENCES

1. Taussig, F. J. Cancer of the Vulva. An Analysis of 155 Cases (1911-1940). Am. J. Obst. & Gynec., **40**:764-779. 1940.
2. Veit, J. Die Erkrankungen der Vulva. Handbuch der Gynäkologie. Dritter Band. pp. 111-255. J. F. Bergmann, Wiesbaden, 1898.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BRYAN, W. R., and M. B. SHIMKIN. [Nat. Cancer Inst., Bethesda, Md.] QUANTITATIVE ANALYSIS of DOSE-RESPONSE DATA OBTAINED WITH CARCINOGENIC HYDROCARBONS. J. Nat. Cancer Inst., 1:807-833. 1941.

Statistical methods developed for the analysis of dose-response data are discussed and applied to problems involving the responses of animal groups to the action of carcinogenic hydrocarbons. The authors feel that the application of these biomathematical methods allow: (1) a clear and concise presentation of the experimental data; (2) an estimation of the statistical significance and reproducibility of the data; (3) an interpolation of data, within the range of observed results, without further experimentation; and (4) the guidance of further experimentation by the extrapolation of the available data.—L. L. W.

COWDRY, E. V., and F. X. PALETTA. [Washington Univ. and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] CHANGES IN CELLULAR, NUCLEAR, AND NUCLEOLAR SIZES DURING METHYLCHOLANTHRENE EPIDERMAL CARCINOGENESIS. J. Nat. Cancer Inst., 1:745-759. 1941.

The nuclear and cytoplasmic volumes of both basal and spinous cells of mouse epidermis progressively increased in size following the application of methylcholanthrene under standardized conditions. However, these values were markedly decreased in a carcinoma developing from these precancerous hyperplastic cells. Mitoses were more frequent in hyperplastic basal cells than in hyperplastic spinous cells, or in untreated epidermal cells, but were less than in malignant cells.

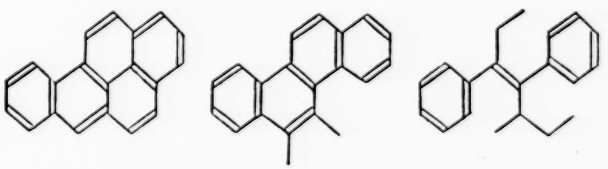
The cells of 17 squamous carcinomas produced by methylcholanthrene were compared with the hyperplastic, nonmalignant cells included in the same sections. No single measurement of size revealed significant differences between malignant and nonmalignant cells. Comparative measurements of the cells of human carcinomas and adjacent hyperplastic epithelial cells likewise revealed no single definite criterion that could be used to differentiate malignant and hyperplastic cells.—L. L. W.

CRAMER, W., and R. E. STOWELL. [Washington Univ. and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] CARCINOGENESIS IN THE MOUSE'S SKIN BY THE INFREQUENT APPLICATION AT LONG INTERVALS OF METHYLCHOLANTHRENE. Cancer Research, 1: 849-852. 1941.

Cancer has been induced in the skin of Swiss mice by a method of application in which the carcinogen acts on the cells infrequently and at long intervals. A 0.6% solution of methylcholanthrene in benzene was applied at intervals of 2 weeks, 3 weeks, and 1 month, respectively. Using this protracted technic, the dose of carcinogen effective in producing cancer was smaller than when it was applied thrice weekly for 14 weeks. Furthermore, the dose became increasingly smaller as the interval between successive applications was prolonged. These results are not in accordance with the accepted view that the carcinogenic hydrocarbons induce cancer in the skin by stimulating directly the mitotic activity of the epithelium. They support a conception, arrived at from a histological study of the early changes in carcinogenesis, that the carcinogenic hydrocarbons produce a transient toxic effect on the epithelium, inhibiting mitotic activity, and that the epithelial proliferation which eventually leads to cancer is due to the formation in the skin of a substance stimulating the epithelial cells to mitotic activity for a prolonged period of time. The significance of these findings in relation to human skin cancer is discussed.—Authors' abstract.

DODDS, E. C., W. LAWSON, and P. C. WILLIAMS. [Courtauld Inst. of Biochemistry, Middlesex Hosp. Med. Sch., London] CARCINOGENIC AGENT WITHOUT THE CONDENSED CARBON RING STRUCTURE. Nature, London, 148:142. 1941.

In an earlier publication (Dodds, E. C., L. Golberg, W. Lawson, and R. Robinson, Nature, London, **141**:247, 1938) the formula of the synthetic estrogen, stilbestrol, was shown to suggest that ring closure might produce compounds similar to estrone or to chrysene. Since all the hitherto known carcinogenic hydrocarbons show the condensed ring structure, a compound was sought for in which the carcinogenic power would be retained when the ring structure was opened. α-Ethyl-β-secondary-butylstilbene, which is related to 3,4-benzpyrene and to 1,2-dimethyl chrysene, was applied in benzene to the skin of 50 mice, and two tumors (one spindle cell epithelioma and one sarcoma, without metastases) were obtained after 12 and 15 months, respectively. The sarcoma arose on the back 2 cm. behind the painted area, and is growing in the first grafted generation.



Benzpyrene | Dimethylchrysene | α-Ethyl-β-*sec*-Butylstilbene

No tumors of the skin were obtained in periods of 9 months or less with diphenylhexane, diphenylhexadiene, diethylstilbene, 4,4′-dihydroxy-αβ-diethylstilbene (stilbestrol), 4,4′-dihydroxydiphenyl-hexane (hexestrol), or 4,4′-dihydroxystilbene. Stilbestrol given by this method was toxic and none of the mice receiving it lived for more than 3 months.—E. L. K.

KLEINENBERG, H. E., S. A. NEUFACH, and L. M. SCHABAD. [All-Union Inst. for Exper. Med., Leningrad, U. S. S. R.] FURTHER STUDY OF BLASTOMOGENIC SUBSTANCES IN THE HUMAN BODY. Cancer Research, 1: 853-859. 1941.

Blastomogenic (carcinogenic) substances were extracted by benzol from the human lung as well as from the liver. The lungs from which blastomogenic extracts were made

were obtained from patients dying of cancer and also from patients dying of other diseases. The extracts produced a variety of tumors at the sites of injection and at other locations when injected subcutaneously into mice of the R. V. strain and in mice of a strain of unknown origin. The extracts of lungs were less toxic than extracts of liver. The results confirm and extend previous reports of these authors. The possible origin of the blastomogenic substances in the lung from exogenous or endogenous sources is discussed.—S. B-J.

LARIONOW, L. TH. [Central Roentgenological, Radiological, and Cancer Inst., Leningrad, U.S.S.R.] ON THE MECHANISM OF ACTION OF CARCINOGENIC SUBSTANCES. Cancer Research, 1: 860-868. 1941.

General systemic effects of carcinogenic hydrocarbons (benzpyrene) applied to the skin of mice were investigated by determinations of the ratios of partially oxidized substances excreted in the urine, the oxidation-reduction potentials of the blood, and the oxygen consumption of slices of organs. Local effects upon oxidation processes were studied in tissue slices and in skin to which the hydrocarbons had been applied. The oxidation processes were not disturbed during the precancerous period of papilloma formation, but supervened in a secondary manner after the appearance of carcinomas.

Incomplete investigations of proteins of induced tumors indicated that they contained an abnormally large proportion of d-glutamic acid.

The inclusion of the carcinogenic hydrocarbons, benzpyrene and dibenzanthracene, in the medium of tissue cultures of mouse fibroblasts produced changes in the morphological, biochemical, and proliferative characteristics of the cells. The data were not sufficient to indicate whether these cells had been transformed into malignant cells.

Traumatization of the nervous system by section of the sciatic nerve and treatment of the central end with formalin or croton oil affected the incidence of induced carcinoma in mice. An accelerating or inhibiting effect was dependent upon the time in the experimental cycle at which the lesions of the nervous system were produced.

The following conclusions are suggested: 1. Carcinogenic hydrocarbons applied to the skin of mice do not affect the oxidation processes during the precancerous period of papilloma formation. 2. Changes in the oxidation processes of the organism of some organs, and of tissues occurring in connection with carcinoma induced by carcinogenic hydrocarbons are of a secondary nature. 3. The primary change caused by carcinogenic hydrocarbons may be an alteration of protein metabolism. 4. Carcinogenic hydrocarbons produce changes in cells by action directly upon the cells. 5. In the organism the nervous system seems to function as an intermediary link in the production of carcinomas induced by carcinogenic hydrocarbons.—Author's summary as revised by S. B-J.

SHIMKIN, M. B. [Nat. Cancer Inst., Bethesda, Md.] LENGTH OF SURVIVAL OF MICE WITH INDUCED SUBCUTANEOUS SARCOMAS. J. Nat. Cancer Inst., 1:761-765. 1941.

The length of time between the detection of induced subcutaneous sarcomas by palpation and death of the animal was studied in male mice of strains C3H and L. The mean length of survival, 4.36 ± 0.10 weeks, was the same whether 20-methylcholanthrene or 1,2,5,6-dibenzanthracene was used, whether the dose of the carcinogen was 0.1 or 0.5 mgm., and whether palpable tumors appeared early or late following the injection of the carcinogen.—Author's summary.

WOGLOM, W. H. [Columbia Univ., New York, N. Y.] NECROSIS AND SARCOGENESIS. Am. J. Cancer, 40:429-430. 1940.

In an attempt to assess the significance of necrosis in the pathogenesis of neoplasia, 144 Dobrovolskaïa-Zavadskaïa R III strain mice were injected subcutaneously four times at intervals of 4 weeks with 0.05 cc. of minced spleen from animals of this same strain. One week after each of these treatments 0.1 mgm. of methylcholanthrene in 0.05 cc. of olive oil was injected at the same site. An equal number of controls receiving methylcholanthrene alone were prepared. After correcting for extraneous mortality and ulceration at the site of injection, 82% of the controls bore tumors. Latent periods and rate of tumor growth were approximately the same. It is therefore concluded that the presence of necrotic splenic tissue had no effect on the incidence or growth characteristics of the tumors produced.—L. L. W.

Hormones

CRAMER, W. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] THE PREVENTION OF SPONTANEOUS MAMMARY CANCER IN MICE BY AN ANTERIOR PITUITARY HORMONE. Am. J. Cancer, 40:431-433. 1940.

This paper compares and reanalyzes the data of Cramer and Horning (Lancet 1:72. 1938) and Haagensen and co-workers (Proc. Soc. Exper. Biol. & Med., 45:820. 1940). The conclusion drawn is that both experiments, when carefully examined, show the treatment of Paris R III mice with anterior pituitary hormone to be effective in preventing the development of spontaneous mammary cancer.—L. L. W.

Viruses

DAUGHADAY, W. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] A COMPARISON OF THE X-ZONE OF THE ADRENAL CORTEX IN TWO INBRED STRAINS OF MICE. Cancer Research, 1: 883-885. 1941.

Observations were made on the adrenals of two strains of mice, the Dba strain which has a high incidence of mammary tumors, and the C57 black strain in which mammary tumor incidence is low. Virgin females, males, castrated females, and castrated males were used. Castrations were performed at 1, 21, and 41 days of age. A difference between the x-zone of virgin females of the Dba and C57 black strains was noted. The x-zone of the Dba mice persists for over 200 days and its regression is accompanied by vacuolization. The x-zone of the C57 mice on the other hand undergoes complete regression by 100 days without vacuolization. The x-zone of the male and female mice castrated before puberty resembles the virgin females of the same strain. The x-zone of the hybrid between the Dba and the C57 strains resembles the JAX Dba parent. Hybrids having the C57 mother seem to show a larger x-zone than those having a Dba mother.—Author's abstract.

Genetics

ANDERVONT, H. B., and W. J. McELENEY. [Nat. Cancer Inst., Bethesda, Md.] SPONTANEOUS TUMORS IN A SUBLINE OF STRAIN C3H MICE. J. Nat. Cancer Inst., 1:737-744. 1941.

The incidence of spontaneous tumors of the mammary gland, liver, and lung in a subline of strain C3H mice maintained at the National Cancer Institute is reported. Of 1,558 breeding females, 91.37 per cent developed spontaneous mammary gland tumors at an average age of 8.58 months. Of 350 virgin females, 97.43% developed spontaneous mammary gland tumors at an average age of 10.43 months. Of 141 females over 1 year of age, 9.95% developed spontaneous hepatomas and 4.25% developed pulmonary tumors. Of 320 males over 1 year of age, 26.87% developed spontaneous hepatomas and 7.81% developed spontaneous pulmonary tumors.

This subline of strain C3H mice is highly susceptible to spontaneous mammary gland tumors and spontaneous hepatomas but relatively resistant to spontaneous pulmonary tumors. Other types of spontaneous neoplasia are uncommon. This colony is regarded as a separate line of C3H mice, and results obtained with it are not directly comparable with those procured with other lines of strain C3H mice.—From author's summary.

GROSS, L. [Christ Hosp., Cincinnati, Ohio] THE INFLUENCE OF SEX OF MICE ON ACQUIRED RESISTANCE TO A TRANSPLANTABLE SARCOMA. Cancer Research 1: 880-882. 1941.

Male and female mice, in which tumors produced by intradermal inoculation of sarcoma S 37 disappeared spontaneously, were reinoculated intradermally or intraperitoneally with the same tumor. Practically all females were resistant to reinoculation, whereas one-fourth of the males developed tumors.—Author's summary.

LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] A REVIEW OF PROGRESS IN THE STUDY OF THE GENETICS OF SPONTANEOUS TUMOR INCIDENCE. J. Nat. Cancer Inst., 1:727-736. 1941.

This valuable review covers the development of genetic research on cancer incidence in human and animal material. Certain types of animal tumors considered separately are mammary epithelial tumors, epithelial pulmonary tumors, nonepithelial tumors, and leukemias. The author points to the evidence that general factors such as coat color and hybridization may, in certain cases, influence the incidence of spontaneous tumors in mice. The bibliography includes 82 references.—L. L. W.

ORBISON, J. L., H. A. DAVENPORT, F. B. QUEEN, D. D. SPICER, and R. M. GALT. [Northwestern Univ. Med. Sch., Chicago, Ill.] AN EFFECT OF HEREDITY ON THE SUSCEPTIBILITY OF RATS TO IMPLANTS OF AN INDUCED SARCOMA. Cancer Research, 1: 891-895. 1941.

A group of 46 induced sarcomas obtained by the subcutaneous injection of 20-methylcholanthrene and 9,10-dimethyl-1,2-benzanthracene were transplanted into rats of heterogeneous ancestry. Over half of the tumors were lost by failure to grow in the first passage, and the remainder showed marked variability in the number of takes and the rate of growth. One tumor, a fibrosarcoma (No. R-9), was selected on the basis of transplantability, since it had given about 55% of takes in the first four passages. During subsequent passages of this tumor, susceptibility and resistance to it tended to be manifested in litters and was not a haphazard distribution in individual rats. Separation of the colony into two strains was made by inbreeding animals from susceptible or resistant litters. Of the 263 resistant-strain rats receiving transplants, 71 (27.0%) grew tumors, and of the 1,038 implants made into these animals 187 (18.0%) grew. However, of the 218 susceptible-strain rats into which transplants were made, 210 (96.8%) grew tumors, and of the 892 transplants made, 823 (92.3%) grew. The response observed was hereditary, but was obtained at once by selection of litters rather than by progressive selective breeding.—Authors' summary.

STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] CHEMICAL STUDIES OF THE SUSCEPTIBILITY TO SPONTANEOUS CARCINOMA OF THE MAMMARY GLAND IN MICE. Arch. Path., 32:420-424. 1941.

Tolerance to the lethal effects of salicylaldehyde parallels and may be used as a measure of the intrinsic susceptibility which partly determines carcinoma of the mammary gland in mice.

The genetic factor or factors may exert an influence on the individual prone to have carcinoma of the mammary gland at some future time apparently in two directions: (1) the development of the physiologic state of the organism of which tolerance to the lethal effects of salicylaldehyde may be an index and (2) the physiologic state of the mammary gland as influenced by hormones.—Author's summary.

STRONG, L. C., and W. L. WILLIAMS. [Yale Univ. Sch. of Med., New Haven, Conn.] A GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE. III. LOCAL AND REMOTE INDUCTION OF CARCINOMA OF THE MAMMARY GLAND. Cancer Research, 1: 886-890. 1941.

In a series of 800 mice of the NH descent injected with 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil at 60 days of age, 45 showed definite evidence of carcinoma of the mammary glands. These carcinomas all occurred in female mice only. In addition to these neoplasms, hyperplasia of mammary tissue and squamous metaplasias of mammary tumors were also found in the treated animals. These types of mammary tissue response occurred separately and in combination with other types of tumor, such as (1) spindle cell sarcoma, (2) carcinoma of the skin, and (3) rhabdomyosarcoma. Mice of the NH descent are characterized by showing a high resistance to spontaneous tumors of mammary origin.—Authors' abstract.

Physical Factors

SUGIURA, K. [Memorial Hosp., New York, N. Y.] THE EFFECT OF HIGH AND LOW BODY TEMPERATURES UPON THE GROWTH OF IRRADIATED MOUSE SARCOMA 180. Radiology, 37:85-93. 1941.

Mice bearing transplanted sarcoma 180 were exposed to high and low body temperatures with and without previous roentgen irradiation. Temperature changes alone had little effect on the number of takes or regressions although tumors grew more slowly in mice living in an environment at 4° C. The number of regressions was increased when the tumors were exposed to x-rays and further increased when this was followed by daily bouts of fever induced by ultra short radio waves. The number of regres-

sions was decreased when x-radiation was followed by exposure of the host to a cold environment.

Tumor fragments kept frozen *in vitro* at −70° C. for 7 days showed good growth capacity on subsequent transplantation.—C. E. D.

Radiation

HENSHAW, P. S. [Nat. Cancer Inst., Bethesda, Md.] THE INDUCTION OF MULTIPOLAR CELL DIVISION WITH X-RAYS AND ITS POSSIBLE SIGNIFICANCE. Radiology, 36:717-724. 1941.

The author produced multipolar cleavage in the eggs of *Arbacia punctulata* by roentgen irradiation of either the eggs or the sperm before fertilization. Excellent photomicrographs and drawings illustrate the distortion of spindle pattern and the unequal distribution of chromatin. Unequal distribution of genic material during mitosis generally leads to cell death but may result in viable daughter cells with altered characteristics. A somewhat similar change, generally described as a somatic mutation, is often invoked to explain the origin of spontaneous tumors. The evidence for and against this theory is discussed. The burden of evidence supports the view that the induction of cancer involves changes in the hereditary complex of the tumor cells. Environmental factors may affect the incidence of such changes as is shown by Bittner's work on the foster nursing of mice and the induction of tumors by chemical carcinogens. Multipolar cleavage is held to have possible relation to the production of cancer by radiation since it involves modification or loss of hereditary substances in the daughter cells.—C. E. D.

Biochemistry and Nutrition—Chemotherapy

BECK, S., and P. R. PEACOCK. [Roy. Cancer Hosp., Glasgow, Scotland] GASTRO-PAPILLOMATOSIS DUE TO VITAMIN A DEFICIENCY INDUCED BY HEATED FATS. Brit. M. J., 2:81-83. 1941.

Groups of rats and mice were fed with a variety of repeatedly heated fats in addition to an adequate basal diet. Within a year signs of avitaminosis A appeared among the rats, and cases of ulceration and papillomatosis of the forestomach were observed among those that died. Control animals fed with unheated fats and the same basal diet showed no gross pathological alterations. Extracts of the livers of rats fed with lard heated under various conditions in addition to the basal diet contained reduced amounts of vitamin A as compared with controls. Also, the addition of raw carrot to the diet prevented the occurrence of gastric lesions, thus tending to confirm the association between these and the general condition of avitaminosis A.

The authors regard the results as correlating those of Cramer and of Passey with some of the observations made by Fibiger; and they suggest that the induction of papillomatosis in their experiments is related to the presence in repeatedly heated fats of a factor which interferes in some way with the absorption or metabolism of vitamin A.—A. H.

CRAIG, F. N., A. M. BASSETT, and W. T. SALTER. [Boston City Hosp., and Harvard Univ. Med. Sch., Boston, Mass.] ARTIFICIAL BENIGNANCY OF NEOPLASM. VI. OBSERVATIONS ON THE OXIDATIVE BEHAVIOR OF TUMORS, AND HOMOLOGOUS NORMAL TISSUES. Cancer Research, 1: 869-879. 1941.

When normal and malignant homologous tissues from the identical animal host were studied, the malignant form exhibited much lower cytochrome system activity than the benign form. This was true of hepatoma, mammary cancer, rhabdomyosarcoma, and sarcoma 180 in mice. Sarcoma 180, which had been rendered slow-growing through "immunization" of the host, nonetheless reacted like other malignant tissues.

These findings suggested that the method might be used in clinical cases to supplement microscopic diagnosis of human tissue. A few examples were given, involving human skin, human breast, and human leucocytes. The white blood cells from human leukemia reacted like normal tissues.

Rabbit papilloma induced by the Shope virus behaved like normal skin for several weeks, and then lost much of its cytochrome system activity.—Authors' abstract.

GREENSTEIN, J. P., and W. V. JENRETTE. [Nat. Cancer Inst., Bethesda, Md.] THE DEPOLYMERIZATION OF THYMONUCLEIC ACID BY AN ENZYME SYSTEM IN NORMAL AND CANCEROUS HEPATIC AND MAMMARY TISSUES AND IN THE MILK AND SERA OF SEVERAL SPECIES. J. Nat. Cancer Inst., 1:845-863. 1941.

The presence of an enzyme in certain tissues, and in the milk and serums of certain species, which catalyzes the depolymerization of sodium thymonucleate has been established. This was done by studying the effect upon the viscosity and streaming birefringence of the thymonucleate when mixed with various tissue extracts or body fluids. The rate of diminution of these specific properties served as an index of enzymatic activity. Tissues were studied in pairs: (1) transplanted hepatic tumor in rats and normal and regenerating rat livers; (2) spontaneous mammary tumors and lactating and hyperplastic mammary tissues (induced by stilbestrol). All of these tissues contained thymonucleodepolymerase. The hepatic tumor tissue showed less activity than its normal control, but the mammary tumor tissue was more active than either lactating or hyperplastic breast tissue. The enzyme was found to be present in the milk of the rat, mouse, rabbit, and guinea pig but absent in human milk and that of the cow, goat, and mare. It was found in the serum of the mouse, rabbit, dog, and guinea pig, but was absent in human and horse serum.—L. L. W.

LOOFBOUROW, J. R., and L. JOYCE. [Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, Mass.] INCREASED ULTRA-VIOLET ABSORPTION OF CELLS FOLLOWING IRRADIATION WITH ULTRA-VIOLET LIGHT. Nature, London, 148:166. 1941.

Fleischmann baker's yeast (*S. cerevisiae*) was suspended in isotonic salt solution or water, sealed under a quartz coverslip on a quartz slide, and irradiated continuously on a microscope stage with the radiation ($\lambda = 2800$ A.) employed as the microscope illuminant. Photomicrographs were taken at 15-minute intervals. They showed a progressive increase in the ultraviolet absorption of the cells during irradiation.

Since the wave lengths employed were in the range highly absorbed by purines and pyrimidines, the results are interpreted as indicating the production by the injured cells of "nucleic-acid-like materials," which may function

as growth promoting factors ("intercellular wound-hormones").

It is suggested that the release of such substances into a tumor mass and the surrounding tissues following irradiation may be a significant factor therapeutically.—R. J. L.

Cytology

PARSONS, L. D., and F. L. WARREN. [The Roy. Cancer Hosp. (Free), London, England] CELLULAR CHANGES IN THE SPLEEN AND LYMPH GLANDS IN MICE USED FOR CARCINOGENIC AND RELATED EXPERIMENTS, WITH SPECIAL REFERENCE TO THE GIANT CELLS OF THE SPLEEN. J. Path. & Bact., 52:305-321. 1941.

The number of giant cells per unit area of sections of the spleen was estimated in normal mice and in mice bearing (1) subcutaneous grafts of a 1,2-benzanthracene-*endo*-succinate tumor, (2) subcutaneous grafts of Mal. sarcoma, (3) intraperitoneal grafts of Mal. sarcoma, (4) primary sarcomas from grafted lymph glands, (5) primary Mal. sarcomas, (6) primary Mal. filtrate tumors. Spleens of x-radiated mice and of mice under treatment with the *endo*-succinate of 1,2,5,6-dibenzanthracene were also examined.

In mice bearing subcutaneous grafted sarcomas the splenic giant cells are increased to two or three times their normal number, in contrast to mice bearing intraperitoneal grafts, in which these cells are decreased to one-sixth of their normal number. Mice bearing primary induced sarcomas or receiving injections of a carcinogenic compound show about half the normal number of giant cells. X-radiation greatly diminishes the number of giant cells in the spleen.

A significant degree of correlation was found between the blood leucocyte counts and the splenic giant cell counts in mice bearing subcutaneous grafted sarcomas or primary induced sarcomas.

Extramedullary myelopoiesis, and changes in the lymphoid tissue of the spleens of mice bearing tumors and under other conditions are described.

The induction of 2 sarcomas by injection of the *endo*-succinate of the relatively noncarcinogenic hydrocarbon 1,2-benzanthracene with concurrent x-radiation of the mice is reported.—F. L. W.

Miscellaneous

SELBIE, F. R. [Middlesex Hosp., London, England] A TRANSPLANTABLE MAMMARY FIBROADENOMA OF THE RAT SHOWING SARCOMATOUS CHANGES. Brit. J. Exper. Path., 22:156-166. 1941.

This tumor strain was obtained by Gye from a spontaneous tumor. A full account is given of the changes in transplantability, rate of growth and histological structure in successive generations. The original tumor was transplanted more readily in female (46%) than in male (28%) rats; when the sarcomatous transformation took place this sexual difference ceased. The acini showed secretory activity during pregnancy. Squamous metaplasia occurs, especially in male rats, and sebaceous metaplasia was observed in 3 female rats. There is a tendency for fibrous tissue to outgrow the epithelial cells of these adenomas, and this may continue until the epithelium has wholly disappeared and the tumor takes on the appearance of a fibroma or sarcoma, which is transplantable, but none of these tumors have shown invasion of normal tissues or formation of metastases.—E. L. K.

Clinical and Pathological Reports

Etiology

FORSTER, N. K. [Hammond, Ind.] PRESENT DAY ASPECTS OF THE ETIOLOGY OF CANCER. J. Indiana M. A., 34:208-210. 1941.

This is a synopsis of the available experimental data on the etiology of malignant disease, so designed as to be of interest to the general physician.—M. J. E.

LEVINSON, L. J., and N. J. FURST. [Newark Beth Israel Hosp., Newark, N. J.] TRAUMA ASSOCIATED WITH MALIGNANCY. J. M. Soc. New Jersey, 38:181-184. 1941.

Two cases illustrating a possible relationship of trauma to malignant tumors are recorded. A patient was struck on the thigh with a 100-pound container of white lead. Some swelling resulted after 2 weeks, but 9 months later the part was invaded by an extensive synovial sarcoma which metastasized to the lungs. A second patient, a man with a probably antecedent mammary fibroadenoma, received a lacerating injury of the breast. Six months later the pectoral region was infiltrated by an adenocarcinoma, possibly originating in the earlier tumor. Two examples of certain nonassociation of trauma and malignant disease are appended.—M. J. E.

Heredity

BELL, J. A DETERMINATION OF THE CONSANGUINITY RATE IN THE GENERAL HOSPITAL POPULATION OF ENGLAND AND WALES. A COLLABORATIVE STUDY SUMMARIZED BY THE AUTHOR. Ann. Eugenics., 10: 370-391. 1941.

This paper is concerned with facts relating to consanguinity in marriage deduced from an examination into the parentage of in-patients of general hospitals and neurological and cancer hospitals in England and Wales. The consanguinity rate amongst the parents of sufferers from cancer, considered as a single disease, is no greater than has been noted amongst the total of parents of the general hospital population. When, however, the parental consanguinity rate is analyzed for different types of cancer the figures for cancer of the uterus are particularly striking. Thus the percentages of first cousin and consanguineous marriages amongst the parents of the general hospital population are 0.606 and 0.789, respectively, but for cases of uterine cancer, 1.509 and 1.724, respectively. These figures are suggestive of a recessive factor being operative.—R. J. L.

EDMONDS, H. W., and J. W. HAWKINS. [Harvard Med. Sch., Boston, Mass.] THE RELATIONSHIP OF TWINS, TERATOMAS AND OVARIAN DERMOIDS. Cancer Research, 1: 896-899. 1941.

The incidence of twinning, whether measured by the percentage of families with twins or by the ratio of twin births to total births, is similar and consistently high in families of patients with ovarian dermoids, of patients with

childhood teratomas, and of patients with twin pregnancy —higher in each instance than in random control groups. This evidence supports a theory of similarity in pathogenesis of teratomatous tumors and of twins, probably involving common factors of heredity.—Authors' abstract.

GARLAND, A. [Burton Road Hosp., Lincoln, England] FOUR BROTHERS WITH NEUROFIBROMATOSIS. Brit. M. J., 2:120. 1941.

A short history is reported of a family with 4 brothers all suffering from neurofibromatosis of the skin and subcutaneous tissues. One died at the age of 29 having developed paraplegia following spinal involvement. The 3 living are of small stature and their ages range from 50 to 60 years. In 2 of the brothers the tumors have a generalized distribution, but in the third the neck and forehead are alone affected. Of 4 other children of the same family one is feebleminded. The parents are dead and there is no record of their having suffered from neurofibromatosis. The father contracted a second marriage, which resulted in 4 normal children. Hence the author suggests that the disease was transmitted by the first wife as a Mendelian dominant trait.—R. J. L.

Diagnosis—General

MAVER, M. E., J. M. JOHNSON, and J. A. THOMPSON. [Nat. Cancer Inst., Bethesda, Md.] THE *d*-PEPTIDASE ACTIVITY OF SERUM AS AN ALLEGED DIAGNOSTIC TEST FOR CANCER. J. Nat. Cancer Inst., 1:835-843. 1941.

Although the serums of 19 carcinoma patients exhibited considerable *d*-peptidase activity, only one digested more than 50% of the racemic dipeptide, dl-leucylglycine. Thus the work of Waldschmidt-Leitz and Mayer (Ztschr. f. physiol Chem., **262**:IV-VI. 1939.), who used greater than 50% *d*-deptidase activity of serum as a diagnostic test for cancer, was not confirmed. Control serums did not differ greatly from those of cancer patients in *d*-peptidase activity. The peptidase activity of various transplantable animal tumors and the serums of these animals were also studied. No diagnostically useful characteristics were found.—L. L. W.

PHILLIPS, R. B. [Rochester General Hosp., Rochester, N. Y.] THE SYMPTOMS AND SIGNS OF METASTATIC CANCER. Am. J. Surg., 53:486-489. 1941.

Clinical report of obscure cases that turned out to be metastatic cancer.—H. G. W.

Therapy—General

BAUMEISTER, C. F., SR., and C. F. BAUMEISTER. [Avoca, Iowa and San Jose, Calif.] SPONTANEOUS RECESSION OF MALIGNANT TUMORS. J. Iowa M. Soc., 31:106-110. 1941.

A man of 72 years had a subcutaneous mass attached to the sternum, which grew progressively for 5 months. He refused the suggested treatment, and potassium iodide was administered orally. One week later the lump disappeared completely. It recurred 3 weeks later, increased in size for a time and again receded. The patient then died of pneumonia. At necropsy a residual neoplasm, classified histologically as a lymphosarcoma, was found in the medial portion of the thoracic wall infiltrating the pectoral muscle. Fibrotic transformation similar to that induced by roentgen therapy was prominent in the growth.—M. J. E.

de CHOLNOKY, T. [New York Post-Grad. Med. Sch. and Hosp., Columbia University, New York, N. Y.] ELECTROSURGERY IN ADVANCED CANCER AND RECONSTRUCTION. Arch. Phys. Therapy, 22:21-27. 1941.

Electrosurgical destruction of advanced cancers and removal of the coagulated tissue offer a safe method of obtaining gratifying palliative results in patients who otherwise would be condemned to a trying existence with ulcerated infected tumors. Occasionally an apparently complete cure may be achieved. The technic is simple, and the risk small even in debilitated patients. Two cases are recorded to illustrate the beneficial results of this therapy. The first patient had a recurrent large ulcerated basal cell cancer of the scalp, which infiltrated the skull and exposed the dura. Electrosurgery produced some improvement, but he died of a recurrence 8 months later. The second had a recurrent squamous cell cancer of the thigh with inguinal metastases. Healing followed coagulation of the tumor and dissection of the metastastic deposits, and the patient appeared tumor-free 6 years later. Photographs of the lesions before and after treatment are reproduced.—M. J. E.

NEWMAN, M. K., and J. M. BERRIS. [Detroit, Mich.] ARTIFICIAL HIBERNATION THERAPY. Arch. Phys. Therapy, 22:161-170. 1941.

The body temperature of 11 patients with advanced cancer was lowered to 85-88° F. in a specially constructed room or in individual cooling units for periods of 3 to 5 days. In addition local refrigeration of tumors was maintained in 6 patients for 2 to 3 weeks with the aid of ice water circulating in rubber tubing. Treatment relieved pain.—M. J. E.

Radiation—Diagnosis and Therapy

ALLISON, R. G. [Minneapolis, Minn.] X-RAY THERAPY IN INFECTIONS AND TUMORS. Journal-Lancet, 51:190-192. 1941.

General remarks on the technic employed for various types of infections and neoplastic lesions.—M. J. E.

COWIE, D. B., and L. A. SCHEELE. [Nat. Cancer Inst., Bethesda, Md.] A SURVEY OF RADIATION PROTECTION IN HOSPITALS. J. Nat. Cancer Inst., 1:767-787. 1941.

Fifty-five hospitals were surveyed and approximately 225 persons examined in this study. Inadequate protection against radium and x-rays was found in many institutions and resulted from poor storage, transportation and manipulating facilities, or from indifference or carelessness on the part of those persons exposed. Skin changes due to radiation were found in nearly one-quarter of the radiologists examined. Methods for measuring and reducing exposure are given. The recommended per diem tolerance dose is 0.02 r.—L. L. W.

FRICKE, R. E., and C. O. HEILMAN. [Mayo Clinic, Rochester, Minn.] RESULTS OF RADIUM TREATMENT IN CANCER OF THE UTERINE FUNDUS. J. A. M. A., 117:980-982. 1941.

A study of 109 cases treated with radium alone shows that low grade malignant tumors respond well and offer the best chance of cure. A 5-year cure was obtained in this

series of cases which were considered unfavorable for operation.—H. G. W.

GRUNER, O. C. [Montreal, Canada] PERIODIC FLUCTUATIONS IN THE BLOOD PICTURE IN CANCER AND THEIR BEARING ON RADIATION THERAPY. Canad. M. A. J., 44: 256-259. 1941.

The author supports the view that cancer follows a cyclical pattern, as evidenced by the course of symptoms and their recurrent nature. Renewed manifestations of the disease following successful therapy generally occur after a period of 231 days. In some instances half cycles appear to exist. This observation suggests the presence of an underlying, periodically active exogenous factor. Associated blood abnormalities are considered of diagnostic value. Malignant disease may disclose its presence by the finding in smears of inclusion bodies in monocytes, or bizarre forms of polymorphonuclear leucocytes. The hematologic alterations are likewise cyclical, and may remain masked during negative phases of the general cycle of the disease. Proof of these claims is given in a drawing and a series of graphs.—M. J. E.

HENSHAW, P. S. [Nat. Cancer Inst., Bethesda, Md.] BIOLOGIC SIGNIFICANCE OF THE TOLERANCE DOSE IN X-RAY AND RADIUM PROTECTION. J. Nat. Cancer Inst., 1:789-804. 1941.

The history of protection from injury caused by x-rays and gamma rays is briefly given. This brings out the fact that many biological factors involved in such injuries have been overlooked. Whereas such injuries as tissue destruction and blood changes have threshold dose-effect values, radiogenetic injuries, being the result of single event action, do not. Therefore, while there are safe exposures for the former type of reaction none exist for the latter. The far-reaching biological effects of radiogenetic injuries are stressed.

The tolerance dose (for threshold-type reactions) is defined as the amount of x-ray or gamma ray energy that a person can receive continuously or at intervals without suffering changes in the blood, when the whole body is exposed, or redness of the skin when local areas are concerned. The safety standard of tolerance dose is thought to be somewhere between 0.12 and 0.02 r per day.—L. L. W.

JACOBY, P., and J. SPOTOFT. [Radium Center, Odense, Denmark] INVESTIGATIONS INTO THE SIGNIFICANCE OF THE SEDIMENTATION REACTION AS REGARDS THE PROGNOSIS OF IRRADIATION OF CANCER PATIENTS. Radiology, 36:617-620. 1941.

The authors believe that an increased sedimentation rate is a nonspecific reaction whose cause is to be sought in the parenteral absorption of albuminous substances in inflammatory or necrotic processes. Either an increase or a decrease in rate may follow irradiation of a tumor. In 59 cases of carcinoma of the breast and 33 of uterine and cervical carcinoma the changes in sedimentation rate during radiation therapy were of no prognostic significance. However, follow-up studies, covering 1 to 5 years after treatment, reveal that a persistent or periodic elevation of sedimentation rate is a bad prognostic sign often associated with recurrence or metastases. The same generalization applies to other forms of cancer.—C. E. D.

LITTIG, L. V. [Madison, Wis.] PELVIC BONE METASTASES, FROM CARCINOMA OF THE BREAST, TREATED WITH ROENTGEN THERAPY; A CASE REPORT. Wisconsin M. J., 40:479. 1941.

Extensive pelvic metastases of a carcinoma of the breast appeared 3 years after a radical mastectomy. Recalcification of destroyed bone and clinical improvement followed fractionated roentgen therapy (total dose 5,200 r) administered through 3 portals.—M. J. E.

MARTIN, C. L. [Dallas, Tex.] ADVANCES IN THE TREATMENT OF CANCER WITH RADIATION. J. Tennesee M. A., 34:41-47. 1941.

The author considers radiation to be the method of choice in the treatment of 80% of all cancer patients. General remarks are given on the curative and palliative effects of roentgen therapy for various types of tumors. More specific attention is directed to the technic in cases of cancer of the lip, mouth, or pharynx associated with unilateral or bilateral cervical metastases. A modified Coutard technic is employed for the eradication of the primary neoplasm. Block dissection of the neck has been abandoned, and instead a combined form of therapy with radium needles and fractionated roentgen radiation is utilized. Of 39 patients treated 36 were improved immediately, and 24 survived for 1 year or longer. In the latter group are included 8 patients alive for 4 or more years. The method is advocated as a prophylactic measure when palpable nodes are not detected.—M. J. E.

RINEHART, D. A., and B. A. RINEHART. [Little Rock, Ark.] PROTRACTED ROENTGEN THERAPY OF MALIGNANCIES. South. M. J., 34:820-822. 1941.

Protracted fractional roentgen therapy may be used in malignant tumors about the head and neck whenever other methods of treatment are not applicable.—H. G. W.

ROBINSON, G. A. [New York, N. Y.] PRINCIPLES OF RADIATION THERAPY OF HEAD AND NECK NEOPLASMS. Arch. Phys. Therapy, 22:220-224. 1941.

The author outlines his technic for various forms of cancer. Facial cancer is treated with low voltage unfiltered or lightly filtered roentgen radiation, hemangioma with radium, cancer of the nasal sinuses with fractionated high voltage roentgen rays followed by surgical removal, and cancer of the tongue and tonsil with fractionated roentgen therapy and radium.—M. J. E.

Skin and Subcutaneous Tissues

BLANCHARD, A. J. [Toronto Gen. Hosp., Toronto, Canada] THE PATHOLOGY OF GLOMUS TUMOURS. Canad. M. A. J., 44:357-360. 1941.

Four cases are recorded. The tumors were located in the region of the ankle, upper arm, patella, and nail bed. Excision effected a cure. The histological appearances were typical.—M. J. E.

COUCH, J. H. [Univ. of Toronto, Toronto, Canada] GLOMUS TUMOURS: CLINICAL PICTURE AND PHYSIOLOGY. Canad. M. A. J., 44:356-357. 1941.

A glomus tumor was demonstrable microscopically in the excised nail bed of a patient with the characteristic history of excruciating pain in the subungual region of the involved finger. Because of its tiny dimensions the tumor was not palpable nor seen with the unaided eye after removal of the nail. The operation afforded immediate and permanent relief. A discussion of the thermostatic function of the normal glomus is appended.—M. J. E.

PLEWES, B. [Toronto, Canada] MULTIPLE GLOMUS TUMOURS; FOUR IN ONE FINGER TIP. Canad. M. A. J., 44:364-365. 1941.

Three distinct glomus tumors were successfully excised from the palmar aspect of the right little finger and a fourth from the ulnar side of the root of the finger nail of a patient 16 years old.—M. J. E.

FEDERSPIEL, M. N. [Milwaukee, Wis.] EXTENSIVE PLASTIC REPAIR FOR RECONSTRUCTION OF THE LOWER LIP (REPORT OF A CASE IN WHICH THE LOWER LIP WAS ENTIRELY REMOVED FOR THE ERADICATION OF AN EPITHELIOMA). Wisconsin M. J., 40:289-291. 1941.

Technical details are given for an operation performed in 2 stages to reconstruct the lower lip, previously excised for cancer, by utilizing the soft structures of the lower cheek, chin, and neck.—M. J. E.

FISHBACK, H. R. [Northwestern Univ. Med. School, Chicago, Ill.] GIANT PIGMENTED NEVUS WITH MALIGNANT TRANSFORMATION. Am. J. Cancer, 40:471-473. 1904.

An extensive pigmented nevus of the trunk, present since birth, underwent malignant transformation when the patient was 37 years of age. The malignant change occurred about 4 years after partial operative removal.—L. L. W.

HALFORD, F. J., and H. C. GOTSHALK. [Honolulu, Hawaii] EPITHELIOMATOUS DEGENERATION IN THE SCAR OF A BURN. Arch. Dermat. & Syph., 44:26-29. 1941.

A case in which 2 epitheliomatous ulcers developed in the large scar of a burn received 16 years before, in a man of 21. The first ulcer was cured by excision and skin graft but the second produced rapidly spreading fatal metastasis. The age of the scar, not the patient, is the important factor in cancer arising in burn scars.—H. G. W.

LAMB, J. H., and W. E. EASTLAND. [Oklahoma City, Okla.] CANCER OF THE LOWER LIP. J. A. M. A., 117: 600-607. 1941.

Cancer of the lower lip was treated in 318 cases by roentgen rays and radium. Of these 22 have died or now have incurable metastases (7%). There were 72.2% 5-year survivals. Protracted radium therapy with the element well filtered has produced a higher percentage of cures than did the older technic which employed weaker doses at short intervals. Results obtained by interstitial radiation therapy in a restricted number of patients surviving 5 years or more revealed 95% evidence of cure in the primary lesion.—H. G. W.

NEVE, E. F. [Kashmir Mission Hosp., India] KANGRI-BURN CANCER. Indian M. Gaz., 76:138-140. 1941.

A review of the etiology, gross pathology, histology, and treatment of Kangri-burn cancer. During a period of 50 years, 3,064 operations were performed in the Kashmir Mission Hospital for Kangri-burn epithelioma.—A. H.

NICHOLAS, L. [Univ. of Penn. Graduate Med. Sch., Philadelphia, Pa.] ACANTHOSIS NIGRICANS OVERLYING METASTATIC MALIGNANT GROWTHS OF THE SKIN. Arch. Dermat. & Syph., 44: 349-358. 1941.

A case of acanthosis nigricans is reported in which deep cutaneous and subcutaneous metastases from a retroperitoneal lymphosarcoma were observed histologically.—H. G. W.

REUTER, M. J., and R. NOMLAND. [Milwaukee, Wis., and Iowa City, Iowa] INFLAMMATORY CUTANEOUS METASTATIC CARCINOMA. Wisconsin M. J., 40:196-201. 1941.

Three cases are recorded of the extensive inflammatory type of metastases of carcinoma of the breast in the skin of the chest wall, and one of involvement of the face and neck secondary to a cancer of the rectum. Photographs of patients and photomicrographs are reproduced. The latter demonstrate the basis of the syndrome in the widespread extension of the malignant process in the lymphatic vessels of the skin.—M. J. E.

SCANNELL, R. C. [Carroll, Iowa] SUBUNGUAL MELANOMA. Am. J. Surg., 53:163-167. 1941.

Case report.—H. G. W.

SIMPSON, F. E. [Chicago, Ill.] CANCER AND PRECANCEROUS LESIONS OF THE LIP. Illinois M. J., 79:459-464. 1941.

Following a presentation of the clinical and pathological aspects of cancer of the lip the author records the results of treatment with radon tubes applied externally to the lesions. Of 114 patients with carcinoma, 10 had cervical metastases, and 101 were clinically cured after periods of 3 to 15 years. Photographs illustrate the excellent cosmetic results.—M. J. E.

SPENCER, G. A. [Harlem Hosp., New York, N. Y.] SQUAMOUS CELL EPITHELIOMA ASSOCIATED WITH SENILE KERATOSIS ON THE LEG OF A NEGRO. Arch. Dermat. & Syph., 44:214-217. 1941.

The case is reported of a dark-skinned negress with depigmentation of the legs on which a senile keratosis and squamous cell epithelioma had developed. It is suggested that the disappearance of pigment may have acted as a contributing factor in the development of the malignant growth, as only 1 case of basal cell epithelioma was found in a series of 5,000 cases observed in the negro clientele of the Harlem Hospital, and that was in a mulatto.—H. G. W.

WARREN, S., and C. R. LULENSKI. [Collis P. Huntington Memorial Hosp., Boston, Mass.] END RESULTS OF THERAPY OF EPITHELIOMA OF THE SKIN. Arch. Dermat. & Syph., 44:37-42. 1941.

Analysis of the end results in 451 cases of basal cell epithelioma of the skin. Only in 41 of 543 cases did the lesion appear on the trunk and extremities. Multiple epitheliomas were present in 14% of the patients. The chronicity of this type of lesion is striking, many of them having a span of 10 or more years from onset to final healing, and patients delayed seeking treatment for an average period of 3 to 4 years. The highest proportion of failures (48%) occurred with radon therapy, and the lowest (8%) with surgical treatment. The size of the lesion at the time of the first treatment is of special importance, for treatment failed with only 9% of the epitheliomas 1 cm. in diameter or smaller, as contrasted with 65% failure where the ulcer was over 5 cm. in diameter. Neither the age of the patient nor the location of the tumor had any apparent influence. The mixed basal cell and epidermoid epitheliomas had a lower percentage of cures than the other types of basal cell tumor. The basal cell epitheliomas have a better prognosis from the standpoint of mortality than epidermoid epitheliomas (11% as against 34.4%) but have a very little better outlook as far as 5-year cures are concerned.—H. G. W.

YOUNG, F. [Rochester, N. Y.] THE TREATMENT OF PERSISTENT RECURRENT BASAL CELL CARCINOMA OF THE FACE. Surg. Gynec. & Obst., 73:152-162. 1941.

The majority of basal cell carcinomas are at present treated initially by irradiation, and successfully. But once recurrence occurs following irradiation, radical surgical extirpation should be done. The surgical technic involved is discussed.—H. G. W.

Nervous System

DRAKE, R. L. [Wichita, Kans.] LYMPHOSARCOMA INVOLVING THE EPIDURAL SPACE. J. Kansas M. Soc., 42: 212-222. 1941.

A laminectomy was performed on a patient with a transverse myelitis located clinically in the upper thoracic segments. A lymphosarcomatous mass was excised from the dorsolateral surface of the cord at the level of the second dorsal vertebra. The previous symptoms disappeared completely following roentgen irradiation (total dose 4,000 r). In the succeeding period deposits of lymphosarcoma appeared in the parotid gland (associated with a mixed tumor), over the sacrum, and in the abdominal lymph nodes. Radiation was of temporary value, and the patient died 1½ years after the first operative intervention.—M. J. E.

HAVERFIELD, W. T., and A. E. WALKER. [Univ. of Chicago Clinics, Chicago, Ill.] LIPOBLASTIC MENINGIOMA. Arch. Surg., 42:371-378. 1941.

A case of lipoblastic meningioma in an 8-year-old girl is reported.—G. DeB.

JUROW, H. N. [Cincinnati Gen. Hosp., Cincinnati, Ohio] PSAMMOMATOUS DURAL ENDOTHELIOMA (MENINGIOMA) WITH PULMONARY METASTASIS. Arch. Path., 32:222-226. 1941.

A rare case of metastasis of a mature form of dural endothelioma is reported, the growth also invading the brain, in spite of its benign appearance.—H. G. W.

MEREDITH, J. M. [Univ. of Virginia Hosp. and Sch. of Med., University, Va.] UNUSUAL TUMORS OF THE BRAIN—WITH EMPHASIS ON PATHOLOGICAL AND DIAGNOSTIC PITFALLS. A REPORT OF FIVE CASES. Virginia M. Monthly, 68:319-329. 1941.

Combined clinical and roentgenographic evidence in each case was sufficiently characteristic to enable accurate localization of the tumors. Histologically these comprised ependymoma, respectively of the cerebellum and of the posterior fossa filling the fourth ventricle, meningioma of the parietal lobe and in the middle fossa in the basilar region, and multiple small osteomas embedded in the superficial zone of the cortex of the right cerebral hemisphere. The tumors were excised successfully. It was necessary to remove a recurrence of the basilar meningioma after 8 months. The microscopic appearance of this mass warranted a diagnosis of meningeal sarcoma. Roentgenograms, drawings of the operative procedures, and photomicrographs are reproduced.—M. J. E.

TURNER, O. A., and W. McK. CRAIG. [Mayo Clinic, Rochester, Minn.] OSTEOGENIC SARCOMA OF MENINGEAL ORIGIN. Arch. Path., 32: 103-111. 1941.

An unusual intracranial tumor of meningeal origin is described, in which active osteogenic and osteoclastic processes occurred throughout the tissue in combination with sarcomatous transformation of the intervening fibroblastic connective tissue. From a study of this tumor and a review of the literature, it is suggested that true osteoblastic meningeal tumors are rare and that the osseous tissue in most of the so-called osteogenic tumors diagnosed as meningioma is an example of heteroplastic or vicarious formation of bone, secondary to the changes which cause ossification in other organs and tumors in the body. The criterion for the osteogenic character of these meningeal tumors should be the presence of an active cellular osteoblastic process rather than the mere inclusion of bone in the tumor tissue.—Authors' summary.

WOOLSEY, R. D., and R. M. KLEMME. [St. Louis, Mo.] MENINGIOMA OF CHOROID PLEXUS: A CASE REPORT. J. Indiana M. A., 34:18-20. 1941.

A ventriculographic examination on a patient with suggestive, but indefinite signs of an intracranial neoplasm disclosed a greatly dilated left lateral ventricle displacing the ventricular system to the right. A craniotomy was performed, and a large meningioma incorporating the choroid plexus removed from the medial wall of the involved ventricle posterior to the foramen of Monro. The operation resulted in considerable improvement.—M. J. E.

Eye

RIWCHUN, M. H., and E. De COURSEY. [Army Med. Museum, Buffalo, N. Y.] SYMPATHETIC OPHTHALMIA CAUSED BY NON-PERFORATING INTRAOCULAR SARCOMA. Arch. Ophth., 25:848-858. 1941.

Pain of increasing severity in the left eye of 3 years' duration made necessary enucleation of the affected organ. The entire intraocular portion was filled with a melanoma, probably of choroidal origin. Marked reduction in vision was subsequently produced by a sympathetic ophthalmia in the remaining eye. Extended courses of nonspecific protein therapy were employed, and after a year the condition had regressed sufficiently to make the visual acuity comparable to its earlier state.—M. J. E.

THOMPSON, H. E., and M. H. SCHEELE. [Finley Hosp., Dubuque, Iowa] MELANOMA OF THE CHOROID WITH EXTENSIVE ABDOMINAL METASTASES. J. Iowa M. Soc., 31:110-112. 1941.

The patient died of diffuse abdominal and pulmonary metastases of a choroidal melanoma 4½ years after enucleation of the affected eye.—M. J. E.

Breast

WHITE, J. W. [Scranton, Pa.] MALIGNANT VARIANT OF CYSTOSARCOMA PHYLLODES. Am. J. Cancer, 40: 458-464. 1940.

A tumor of the breast diagnosed as cystosarcoma phyllodes recurred after operation and produced metastases in the opposite breast, mediastinum, and lung, proven by autopsy. Photomicrographs are appended.—L. L. W.

Female Genital Tract

HOGE, R. H. [Med. Coll. of Virginia, Richmond, Va.] CARCINOMA OF THE CERVIX: STATISTICAL ANALYSIS OF THE CASES SEEN AT THE MEDICAL COLLEGE OF VIRGINIA. Virginia M. Monthly, 68:39-43. 1941.

During the period of 10 years ending March 31, 1940, 237 verified cases of carcinoma of the cervix were ob-

served. Although white patients constituted the greater number of general hospital admissions, 146 (62%) of those with cervical cancer were negroes. No significant difference was evident in the age distribution in the 2 races. The majority of the tumors were in relatively advanced stages clinically, and of the more malignant types histologically. Syphilis, childbearing, and heredity appeared of no consequence.—M. J. E.

MEYER, R. [Univ. of Minnesota, Minneapolis, Minn.] THE HISTOLOGICAL DIAGNOSIS OF EARLY CERVICAL CARCINOMA. Surg. Gynec. & Obst., 73:129-139. 1941.

Carcinoma of the cervix can be recognized in its early stages from such materials as that obtained at curettage and from the portio. The diagnosis can be made when only superficial epithelium is available for study, and the criteria for such diagnosis are given.—H. G. W.

CHARACHE, H. [Brooklyn Cancer Inst., Brooklyn, N. Y.] METASTATIC CARCINOMA OF THE UTERUS. Am. J. Surg., 53:152-157. 1941.

To the 56 cases reported in the literature of carcinoma of the uterus metastatic from extrapelvic sources, 4 cases are added. Thirty-four or 57.6% had their origin in the breast.—H. G. W.

HABERMEL, J. F. [New Albany, Ind.] EARLY SYMPTOMS AND SIGNS OF CANCER OF THE UTERUS. J. Indiana M. A., 34:211-212. 1941.

General discussion.—M. J. E.

SCHEFFEY, L. C. [Jefferson Med. Coll., Philadelphia, Pa.] PROBLEMS ENCOUNTERED IN THE DIAGNOSIS AND TREATMENT OF UTERINE CANCER. J. M. Soc. New Jersey, 38:120-125. 1941.

The author has discarded surgery since 1924 in the treatment of cancer of the cervix. Fractionated roentgen therapy is now employed as the primary therapeutic method, followed immediately or within several weeks by the insertion of radium capsules in the cervical canal and uterine cavity. Radiations at 200 kv. are administered through 4 portals on the average, each receiving 1,600 to 2,400 r. The dose of radium emanations varies from 3,600 to 5,000 mgm. hrs. Cancer of the fundus is treated by preliminary intrauterine application of radium and subsequent panhysterectomy within 6 to 8 weeks. Between 15 and 20% of patients of the first group, and 25 to 35% in the second are cured.—M. J. E.

FRANK, L. W., and A. J. MILLER. [Louisville City Hosp., Louisville, Ky.] MALIGNANCY OF THE VULVA. Am. J. Surg., 53:412-416. 1941.

Clinical study of 17 cases, with a somewhat lower age incidence than usually reported, and a 5-year curability of about 30%.—H. G. W.

TAUSSIG, F. J. [St. Louis, Mo.] PREVENTION OF CANCER OF THE VULVA. Cancer Research, 1: 901-904, 1941.

Clinical observations on 161 cases of cancer of the vulva, seen in the author's practice during the past 35 years, are analyzed and discussed from the points of view of etiology and prevention. The chief conditions which appear to have etiological importance in cancer of the vulva are urethral caruncle, senile warts, abscess of Bartholin's gland, syphilis, and leukoplakia. This etiologic study points to certain pre-existing lesions tending to the development of malignancy which, if promptly removed, may lead to an appreciable lowering in the incidence of cancer.—Author's summary.

Ovary

GRAYZEL, D. M., and H. H. FRIEDMAN. [Jewish Hosp. of Brooklyn, Brooklyn, N. Y.] BRENNER TUMOR OF THE OVARY. Am. J. Surg., 53:509-511. 1941.

Report of a typical case.—H. G. W.

HENDERSON, D. N. [Toronto, Canada] GRANULOSA AND THECA CELL TUMOURS OF THE OVARY. Canad. M. A. J., 44:20-23. 1941.

A general clinical review containing photographs and photomicrographs of characteristic tumor types.—M. J. E.

KANTER, A. E., and A. H. KLAWANS. [Rush Med. Coll. and Cook County Hosp., Chicago, Ill.] ARRHENOBLASTOMA OF THE OVARY. Am. J. Cancer, 40:474-484. 1940.

A case of arrhenoblastoma of the ovary with teratoma is described in a 32-year-old woman. A slightly increased male sex hormone output in the urine was found. Chemical studies of serum and urine showed low sodium and high potassium in the former and decreased values for these substances in the latter. The differential diagnosis of various types of virilism is briefly discussed.—L. L. W.

Male Genital Tract

HERGER, C. C., and H. R. SAUER. [State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] RELATIONSHIP OF SERUM ACID PHOSPHATASE DETERMINATION TO PRESENCE OF BONE METASTASES FROM CARCINOMA OF PROSTATE. J. Urol., 46:286-302. 1941.

A marked elevation of acid phosphatase in the blood serum is indicative of metastatic bone involvement, and its determination should be a routine measure in all cases of proved or suspected prostate carcinoma. Normal values are not absolute proof of the absence of bone metastases, but bone lesions are less likely. A rapid progression of the acid phosphatase activity is of unfavorable prognostic significance.—H. G. W.

KASHIKURA, K. [Keio-Gijuku Univ.] EIN FALL VON PAPILLÄREM ADENOKARZINOM DES HODENS. [A CASE OF PAPILLARY ADENOCARCINOMA OF THE TESTIS.] Gann, 35:80-84. 1941.

A detailed histological description of a papillary adenocarcinoma of the testicle in a 38-year-old man is reported. The tumor arose from the epithelium of either the rete testis or the tubuli recti.—P. P. C.

McNAMARA, F. P., and H. B. HIBBE. [Finley Hosp., Dubuque, Iowa] CHORIOMA OF THE TESTICLE. J. Iowa M. Soc., 31:160-162. 1941.

A fatal case of chorionepithelioma of the testis with metastases in the spleen, lungs, and lymph nodes of the hilus is recorded.—M. J. E.

VOSBURGH, R. K., and J. E. ALDERMAN. [Syracuse, N. Y.] TESTICULAR TERATOMA METASTASIZING TO THE SPINE. J. Bone & Joint Surg., 23:701-708. 1941.

Report of a case of rare bone metastasis from a teratoma of the testis.—H. G. W.

Urinary System—Male and Female

HYAMS, J. A., and J. M. SILBERBLATT. [Gouveneur Hosp., New York, N. Y.] A CASE OF HEMANGIOMA COINCIDENT WITH PAPILLARY CARCINOMA IN THE URINARY BLADDER. J. Urol., 46:271-277. 1941.

Case report.—H. G. W.

STIRLING, W. C., and J. E. ASH. [Washington, D. C.] PAPILLARY CARCINOMA OF THE KIDNEY. Surg. Gynec. & Obst., 73:305-311. 1941.

A clinical consideration of the hypernephroid tumors of the kidney.—H. G. W.

Oral Cavity and Upper Respiratory Tract

DAVIS, E. D. D. THE CLINICAL ASPECT OF LYMPHOSARCOMA OF THE TONSIL. Proc. Roy. Soc. Med., 34:679-681. 1941.

This condition includes (1) a lymphocytic type and (2) reticular cell sarcoma. Lymphosarcoma of the tonsil is rare, and Davis summarizes the clinical features in 14 cases occurring over a period of 25 years. The paper is followed by a discussion (contributions by R. Scott Stevenson, G. E. Archer, W. A. Mill, and N. E. Negus).—A. H.

Intrathoracic Tumors—Lungs—Pleura

HALPERT, B. [Louisiana State Univ. Sch. of Med., New Orleans, La.] THE INCIDENCE OF CARCINOMA OF THE LUNG. Cancer Research, 1: 900. 1941.

A survey of the necropsy material of the Department of Pathology, University of Chicago, disclosed that during the past decade 74 carcinomas of the lung were discovered in 2,781 necropsies on persons over 1 year old.

As in the necropsy material of the Charity Hospital of Louisiana at New Orleans for the same period, carcinoma of the lung was more than one-half as frequent as carcinoma of the stomach and more frequent than carcinoma of the biliary system and carcinoma of the pancreas together.

The data of this study support the assertion that carcinoma of the lung is becoming the second, if not the first, most common malignant neoplasm in the male.—Author's summary.

Gastrointestinal Tract

DUKES, C. E., and H. J. R. BUSSEY. [St. Mark's Hosp., London, England] VENOUS SPREAD IN RECTAL CANCER. Proc. Roy. Soc. Med., 34:571-573. 1941.

This is a short account, given before the Section of Proctology of the Royal Society of Medicine, of a special study of the state of the veins in rectal cancer. Of 669 operation specimens examined by a dissection technic, clumps of carcinoma cells were found in the hemorrhoidal veins in 111 (16.6%). "Evidence of growth within the hemorrhoidal veins in an operation specimen is an indication that venous spread would certainly have taken place eventually if the operation had not been performed but is not to be taken as proof that spread to the liver or lungs has already occurred."—A. H.

FULLER, R. H. [Univ. of Cincinnati, Cincinnati, Ohio] NEURILEMMOMA OF THE STOMACH WITH PEPTIC ULCER. Arch. Path., 32:441-445. 1941.

A case is reported of neurilemmoma of the stomach complicated by ulceration of the overlying mucosa with erosion of the tumor. This is presented as an instance of peptic ulceration secondary to a benign tumor, and offers support to the theory that peptic ulceration also occurs in carcinoma of the stomach.—H. G. W.

GOOD, C. A. [Mayo Clinic, Rochester, Minn.] TUMEFACTIVE LESIONS OF THE SMALL INTESTINE. J. A. M. A., 117:923-926. 1941.

A plea for more thorough roentgenologic examination of the small intestine, which may reveal the rare tumors of this segment of the bowel.—H. G. W.

Liver

BERMAN, C. [Univ. of the Witwatersrand, Johannesburg, South Africa] THE PATHOLOGY OF PRIMARY CARCINOMA OF THE LIVER IN THE BANTU RACES OF SOUTH AFRICA. S. Afr. J. Med. Sci., 6:11-26. 1941.

This paper is the third of a series dealing with primary cancer of the liver, which makes up over 90% of the cancers occurring among the male Bantu in the Witwatersrand Gold Mines. The two earlier papers described the geographical distribution of this form of cancer and the clinical features of the disease in the Bantu (For abstracts see: Cancer Research 1:176, 177, 1941). The present paper describes, and illustrates with 21 photographs, the morbid anatomy of 54 cases and the microscopic appearances seen in 25 of these; in 34 both lobes were involved, in 19 the right lobe only, and in one the left lobe only were affected. The cancerous liver may weigh 7.1 kg. (normal Bantu liver 1.75 kg.). Twenty-four cases showed hepatocellular and one cholangiocellular cancer; all these livers showed intralobular cirrhosis. In the former the cells were arranged in compact finger-like process which anastomosed or ended freely in blunt rounded extremities; the nuclei were large and often contained multiple nucleoli; and giant cells were numerous. The cholangioma consisted of villi covered by columnar epithelium and lying in cystic spaces; in other parts of the liver there were large numbers of new bile ducts. The author thinks that the hepatomas are unicentric rather than multicentric in origin. Liver cells adjacent to invading cancer zones often showed "collateral hyperplasia." There were extrahepatic metastases in 31 out of 54 cases; the distribution of these is described.—E. L. K.

Bone and Bone Marrow

HOWES, W. E., and S. G. SCHENK. [Brooklyn, N. Y.] ROENTGENOLOGIC CONSIDERATIONS IN THE DIAGNOSIS AND TREATMENT OF PRIMARY MALIGNANT BONE TUMORS. Radiology, 37:18-34. 1941.

Forty cases of proved primary malignant tumors of bone are presented from the standpoint of histological type, location, age and sex distribution, clinical behavior, diagnosis, treatment, and end results. Roentgenographic diagnosis agreed with pathologic diagnosis in 33 of 39 cases. Treatment was by surgery, radiation, or both. Thirteen of the 40 patients were alive at the time of writing but only 3 of these had been followed more than a year after treatment. Average survival from initial symptoms was 16.9 months in 23 fatal cases. The authors are disappointed in the results of radical surgery and believe that the best palliative results are obtainable with radiation therapy often in conjunction with surgery.—C. E. D.

LEUCUTIA, T., E. R. WITWER, and G. BELANGER. [Harper Hosp., Detroit, Mich.] LATE RESULTS IN BENIGN

GIANT CELL TUMOR OF BONE OBTAINED BY RADIATION THERAPY. Radiology 37:1-17. 1941.

Since the benign character of most giant cell tumors of bone has been recognized, conservative therapy has largely replaced radical surgery. A review of the literature on radiation treatment of this condition reveals meager case material and dubious conclusions. The authors studied 33 cases treated at the Harper Hospital from 1923 to 1935. Prior to 1930 most cases were treated by curettage and postoperative irradiation but since then irradiation has been the treatment of choice. Surgical intervention carries the disadvantages of exposure to infection, delayed bone regeneration, and possible induction of malignant changes. Radiation has proved effective in restoring function in all of 15 cases in which no other form of therapy was used.

Among 13 patients treated by curettage and postoperative irradiation, subsequent amputation was necessary in 3 and fatal malignant degeneration occurred in a fourth.

Radiation is administered in an initial dose of about 600 r given to as many fields as is necessary to attain a uniform tumor dose. Two months later, treatment is repeated with a 10% reduction of dose. Progressively smaller doses are given at increasing intervals thereafter until fairly good reossification of bone is obtained. This may require as long as 2 years. Smaller doses at longer intervals are employed in treating children. Supporting casts delay reossification and are not used except in cases of pathological fracture.

Histories of the 33 cases are summarized in tabular form. The paper is illustrated by photographs and numerous roentgenograms. The bibliography contains 23 references. Some differences of opinion appear in a discussion by Drs. J. A. Dickinson, R. H. Stevens, M. Lenz, R. R. Newell, and T. Leucutia.—C. E. D.

PERILLO, J. A. [Edward J. Meyer Memorial Hosp., Buffalo, N. Y.] MULTIPLE MYELOMA; REPORT OF AN UNUSUAL CASE. Radiology, 36:741-743. 1941.

A case is reported of multiple myeloma involving practically all the bones in the body of a 25-year-old man. Features pointed out as interesting are the youth of the patient, the absence of Bence-Jones protein in the urine, elevation of blood phosphatase, and a normal serum protein associated with high total calcium.—C. E. D.

Blood Vessels

LAIRD, E. G. [Wilmington, Del.] DEEP CAVERNOUS HEMANGIOMA OF THE NECK. Am. J. Surg., 53:158-162. 1941.

A case is reported, with reference to 13 similar cases in the literature.—H. G. W.

WINER, L. H. [Minneapolis Gen. Hosp., Minneapolis, Minn.] HEMANGIOMAS, CLASSIFICATION AND TREATMENT. Journal-Lancet, 51:168-172. 1941.

A general clinical discussion is given. As a curative measure either radium or the combined treatment by injection of sclerosing chemicals as 95% alcohol or quinine compounds, and refrigeration or cauterization is advocated.—M. J. E.

Leukemia, Lymphosarcoma, Hodgkin's Disease

COMBES, F. C., and S. M. BLUEFARB. [Bellevue Hosp. New York, N. Y.] GIANT FOLLICULAR LYMPHADENOPATHY. Arch. Dermat. & Syph., 44:409-419. 1941.

The association of this condition and its polymorphous cell sarcoma derivative with lesions of the skin is discussed on the basis of 15 cases in which various clinical diagnoses of cutaneous conditions were made. It is recommended that all patients exhibiting chronic eczematoid dermatitis with lymphadenopathy have biopsies of the lymph nodes, as this syndrome in its early stages is amenable to radiation.—H. G. W.

FEIN, M. J. [New York Post-Graduate Hosp. and Med. Sch., Columbia Univ., New York, N. Y.] LYMPHO-EPITHELIOMA OF THE PAROTID GLAND. Am. J. Cancer, 40: 434-440. 1940.

A case of lympho-epithelioma in a woman aged 28 years is described. A discussion of the origin of the term "lympho-epithelioma" together with a description of "lympho-epithelial organs" is given.—L. L. W.

GIERE, C. N. [El Paso, Tex.] LYMPHOSARCOMA DIAGNOSED GASTROSCOPICALLY. J. A. M. A., 117:173-175. 1941.

Case report.—H. G. W.

SPRONG, A. A. [Sterling, Kans.] RADIATION OF LEUKEMIA. J. Kansas M. Soc., 42:102-105. 1941.

The author discusses the results of roentgen irradiation in 18 cases of myeloid and 2 of lymphatic leukemia. Radiation was administered locally to involved areas in doses of 135 to 300 r 1 to 3 times weekly dependent upon the leucocyte level in the circulating blood. The average duration of life after the onset of treatment was 2.7 years. This is considered longer than the life expectancy in untreated patients. Irradiation also produced considerable subjective improvement.—M. J. E.

MEYER, K. A., L. AMTMAN, and L. PERLMAN. [Cook County Hosp., Chicago, Ill.] ISLET CELL TUMORS OF THE PANCREAS. J. A. M. A., 117:16-20. 1941.

Report of a case of successful removal of an islet adenoma of the pancreas.—H. G. W.

SANO, M. E. [Temple Univ. Sch. of Med., Philadelphia, Pa.] CARCINOMA OF THE TAIL OF THE PANCREAS AND DIABETES. Am. J. Clin. Path., 11:605-616. 1941.

Case report.—H. G. W.

Adrenal

MARTEN, M. E., and L. M. MEYER. [Wyckoff Hts. Hosp., Brooklyn, N. Y.] HEMANGIOBLASTOMA OF THE ADRENAL. Am. J. Cancer, 40:485-487. 1940.

A case of hemangioblastoma of the right adrenal in a 21-year-old woman with multiple colonic polyps and neurasthenia is reported.—L. L. W.

WALSH, J. C., and E. M. MEDLAR. [Schenectady County Tuberculosis Hosp., Schenectady, N. Y., and The Hegeman Research Lab. of the Metropolitan Life Insurance Co., Mount McGregor, N. Y.] ACUTE MYELOGENOUS LEUKEMIA. Am. J. Cancer, 40:447-457. 1940.

A case of acute myelogenous leukemia developing in a subject with pulmonary tuberculosis is presented in detail. Blood smears and counts were available throughout the entire course of the disease. The blood picture at first was that of healing tuberculosis, followed by what appeared

to be an acute monocytic leukemia. The leukemia cells however later differentiated into cells of the myelocytic series. It is concluded that acute myelogenous leukemia may arise directly and not be merely an exacerbation of an unrecognized chronic process. Also it is felt that many cases diagnosed as acute monocytic leukemia are, in reality myelogenous in origin.—L. L. W.

Pituitary

ALBRIGHT, F., W. PARSONS, and E. BLOOMBERG. [Massachusetts Gen. Hosp. and Dept. of Med., Harvard Univ., Boston, Mass.] CUSHING'S SYNDROME INTERPRETED AS HYPERADRENOCORTICISM LEADING TO HYPERGLUCONEOGENESIS. RESULTS OF TREATMENT WITH TESTOSTERONE PROPIONATE. J. Clin. Endocrinol., 1:375-384. 1941.

The authors believe that Cushing's syndrome is due to hyperadrenocorticism with hypergluconeogenesis and decreased availability of amino acids. They infer that the clinical manifestations are the result of protein lack as indicated by muscular weakness with low creatinine excretion, decreased mass of muscles, and thin, friable skin.

Therapy with testosterone propionate in three cases without adrenal cancer resulted in retention of about 20 gm. of nitrogen per 5 days; retention of phosphorus in a ratio to retained nitrogen appropriate to formation of protein; decreased excretion of urinary calcium; delayed rise in serum phosphatase; increase in body weight and strength; decreased redness and friability of the skin.

Contrary to claims in the literature estrogens did not provide useful therapy. Progesterone promoted positive nitrogen and phosphorus balances but was inferior therapeutically to testosterone propionate.—J. B. H.

AORING, C. D., and R. H. FULLER. [Cincinnati Gen. Hosp., Cincinnati, Ohio] CARCINOMA OF THE PITUITARY BODY. CASE RECORD PRESENTING CLINICAL PROBLEMS. Ohio State M. J., 37:350-354. 1941.

An elderly patient with the chief complaint of pain in the frontal region and both eyes presented evidence of bilateral palsy of the 2rd, 4th, and 6th cranial nerves. Involvement of the trigeminal nerve appeared obvious with the history of localized pain. The visual fields remained normal during the course of the illness. Roentgen examination of the skull disclosed a destructive lesion involving the sella turcica, posterior clinoids, clivus, petrous pyramids, and foramen magnum. A biopsy from the sphenoidal sinus suggested malignant disease but the tissue of origin was not identified. Treatment was not attempted. A large basilar, extradural mass was found at necropsy and histologic examination revealed this to be a chromophobic cell carcinoma of the hypophysis. (Cases of this type have been discussed by Weinberger, Adler, and Grant, Arch. Ophth., 24:1197. 1940. as illustrating the cavernous sinus syndrome associated with hypophyseal neoplasms).—M. J. E.

FOSTER, M. A., and J. C. McCARTER. [Univ. of Wisconsin, Madison, Wis.] HYPOPITUITARISM. SIMMONDS' DISEASE ASSOCIATED WITH PERNICIOUS ANEMIA, WITH BIOASSAY OF LARGE CHROMOPHOBE ADENOMA. J. Clin. Endocrinol., 1:436-438. 1941.

Study of a case of Simmonds' disease with pernicious anemia is described. Necropsy revealed a large chromophobe adenoma, which when assayed as 800 mgm. of dried powder, was found to be inactive save for small amounts of the melanophore principle.—J. B. H.

MORAN, T. J., and G. H. FETTERMEN. [Pittsburgh City Home and Hospitals, Mayview, Penn.] CHROMOPHOBE PITUITARY ADENOMA WITH SIMMOND'S DISEASE. J. Lab. & Clin. Med., 26:1289-1294. 1941.

A case of Simmond's disease due to a semicystic chromophobe adenoma which had produced complete destruction of the pituitary body, is reported.—H. G. W.

WEINBERGER, L. M., F. H. ADLER, and F. C. GRANT. [Hosp. of Univ. of Pennsylvania, Philadelphia, Penn.] PRIMARY PITUITARY ADENOMA AND THE SYNDROME OF THE CAVERNOUS SINUS. Arch. Ophth., 24:1197-1236. 1940.

In a not unimportant number of cases the clinical signs of involvement of the structures in the wall of the cavernous sinus occupy either a predominant or important role in the diagnosis of hypophyseal neoplasms. Among 169 cases of verified pituitary adenoma 14 presented evidence of the cavernous sinus syndrome consisting of partial or complete palsy of the 3rd, 4th, and 6th cranial nerves and signs of involvement of the ophthalmic division of the trigeminal nerve. Diplopia arises as a result of the paralysis of the external ocular muscles, while pain is the dominant symptom referable to pressure on the sensory branch of the 5th nerve. A transfrontal craniotomy and an attempt to excise the tumors was performed in all patients in this series. Diagnostic and therapeutic methods are discussed.—M. J. E.

Pineal

GLOBUS, J. H. [New York, N. Y.] PINEALOMA. Arch. Path., 31:533-568. 1941.

Tumors of the pineal region can be identified as members of a single neoplastic group by tracing the pineal body through the several stages of its development and matching a typical section of the tumor under investigation with that of a developing pineal body passing through a given critical histogenetic stage. Realizing that at one stage of the histogenesis of the pineal body ependymal derivatives participate, and recognizing the close relationship between ependymoblasts and spongioblasts, one may reasonably assume that pinealoma may in some instances acquire the spongioblastic variety of pinealoma. The development of precocious puberty is not so common with pineal tumors as is generally assumed, and depends on involvement of the hypothalamus.—H. G. W.

LICHTENSTEIN, B. W. [Univ. of Illinois, Coll. of Med., Chicago, Ill.] TERATOMA OF THE PINEAL BODY. A CLINICOPATHOLOGIC REPORT. Arch. Neurol. & Psychiat., 44:153-161. 1940.

A case report of a large tumor in a female child producing extensive internal hydrocephalus and a fatal termination at the age of 6 months. A photograph of the brain and photomicrographs are included.—M. J. E.

Thyroid

CRAIG, P. E., and C. O. SHEPARD. [Coffeyville and Independence, Kans.] THE DIAGNOSIS OF THYROID MALIGNANCY. J. Kansas M. Soc., 42:51-54. 1941.

In addition to a general survey of cancer of the thyroid the authors record a case of reticulum cell sarcoma involv-

ing this organ. Following an extensive resection of the tumor, fractionated roentgen therapy was administered (6,800 r total dose), but the patient died 2 months later of mediastinal metastases.—M. J. E.

DAVIS, A. C. [Mayo Clinic, Rochester, Minn.] ACROMEGALY. THE THYROID GLAND IN 166 CASES OF ACROMEGALY. J. Clin. Endocrinol., 1:445-449. 1941.

The status of the thyroid gland and basal metabolic rate were recorded in 166 cases of acromegaly. The thyroid was enlarged in about 50% of the cases. The basal metabolic rate was often elevated, averaging +14.7. The basal metabolic rate tended to be highest in cases with adenoma of the thyroid.—J. B. H.

MAYO, C. W., and C. P. SCHLICKE. [Mayo Foundation, Rochester, Minn.] EXOGENOUS TUMORS OF THE THYROID GLAND. Am. J. Path., 17:283-288. 1941.

The infrequency with which metastatic tumors are found in the thyroid gland is noted; 19 such cases from the Mayo clinic are cited. In all but 2 of these the thyroid gland was involved as part of a generalized carcinomatosis. The primary sites were varied, the lung being the most frequent single site. The authors state that 12 of the 19 glands so involved were "frankly adenomatous," concluding that this change may influence the localization and growth of the metastatic cells within the thyroid.—H. B.

TURNER, O., and W. J. GERMAN. [Yale Univ. Sch. of Med., New Haven, Conn.] METASTASES IN THE SKULL FROM CARCINOMA OF THE THYROID. A CLINICAL AND ROENTGENOGRAPHIC STUDY OF TWO CASES WITH A BRIEF SURVEY OF THE LITERATURE. Surgery, 9:403-414. 1941.

Carcinoma of the thyroid metastasizes to bone in about 6% of cases. The skull, vertebrae, and pelvis are the most common sites of osseous involvement. Two cases with massive, pulsating metastases to the skull are presented. In one the metastatic lesion was noted a few months after the thyroid enlargement. She died 3 years after the removal of her large cranial metastasis, and about 5 years after the onset of symptoms. The second case developed skull metastases 5 years after the removal of what was called a "fetal adenoma." An attempt at removal of the metastasis, 8 years after its appearance, was unsuccessful. The bibliography includes 26 papers. There are 7 photographs including 4 roentgenograms.—A. M.

WETHERELL, F. S. [Syracuse, N. Y.] THE DIAGNOSIS AND CURABILITY OF PAPILLARY ADENOCARCINOMA OF THE THYROID GLAND. CASE REPORT. West. J. Surg., 48:707-711. 1940.

Microscopic examination of a small node excised from the subcutaneous tissue at the angle of the jaw of a man of 27 years disclosed a papillary adenocarcinoma. The site of the primary growth was not apparent and small doses of roentgen radiation were administered during the next 14 months. The thyroid was then suggested as the possible origin and a subtotal thyroidectomy disclosed a tumor similar in structure to the previously extirpated node. The patient appeared tumor-free 1 year later. A photograph of the patient and photomicrographs are included.—M. J. E.

Parathyroid

HALL, E. M., and L. CHAFFIN. Univ. of Southern California Sch. of Med. and Surgical Service of Sante Fe Coast Lines Hosp., Los Angeles, Calif.] FINAL REPORT OF A CASE OF MALIGNANT ADENOMA OF THE PARATHYROID GLANDS. West. J. Surg., 48:685-688. 1940.

Four years following resection of a malignant parathyroid adenoma the patient died of pulmonary and osseous metastases. Two photomicrographs are reproduced.—M. J. E.

Thymus

ARONSON, S. F. [Camp Murray, Wash.] MYASTHENIA GRAVIS: A DISCUSSION, WITH PRESENTATION OF A CASE ASSOCIATED WITH A THYMOMA. Ann. Int. Med., 15:137-145. 1941.

To the 88 cases of myasthenia gravis autopsied since 1901 is added another, and in 43 (48%) a lesion of the thymus gland was the outstanding feature. There is a definite causal relationship between the thymus and myasthenia gravis, and roentgen investigation followed by irradiation or surgical removal is the treatment of choice.—H. G. W.

Miscellaneous

BOWERS, W. F. [Omaha, Nebr.] CAROTID BODY TUMOR. A CASE REPORT. Nebraska M. J., 26:142-143. 1941.

In the case described it was necessary to ligate the external carotid artery in order to dissect a benign carotid body tumor from the region of the bifurcation of the common carotid artery.—M. J. E.

CHAPMAN, F. D. [Boston City Hosp., Boston, Mass.] TERATOMA OF THE NECK IN THE NEW BORN. Arch. Path., 32:217-221. 1941.

A case of congenital teratoma of the neck in a male infant is reported. The tumor was a complex teratoma, well encapsulated, and made up of many different types of mature tissue elements.—H. G. W.

COLLINS, N. C., and W. E. ANSPACH. [Sherman Hosp., Elgin, Ill.] FIBROSARCOMA OF PLANTAR TISSUES. Am. J. Cancer, 40:458-464. 1940.

A case of fibrosarcoma of the foot observed over a period of 4½ years is reported. Histological diagnosis was difficult. The tumor became radioresistant after a time, and produced finally pulmonary metastases and death.—L. L. W.

MITCHELL, H. E. [Cleveland, O.] TUMORS OF THE EXTERNAL AUDITORY CANAL, WITH A REPORT OF ELEVEN CASES. Arch. Otolaryng., 32:831-844. 1940.

The series of cases reported by the author includes malignant, benign, and nonneoplastic conditions. Of the 3 patients with squamous cell cancers, 1 was observed in a terminal stage while 2 were subjected to radical operation. A subsequent record was available in only one of the latter cases, and this patient, who received intensive postoperative roentgen therapy, was symptom-free after 1 year. Histologically benign but recurrent cylindroma and keloid are described in 2 patients, and 5 had small osteomatous masses, cured by excision or allowed to remain untreated because of their inconspicuous nature. A final case of nonmalignant ulcer of undetermined etiology is included. A single photomicrograph is reproduced.—M. J. E.